BIOLOGY

OF THE

LABORATORY MOUSE

BY THE STAFF OF

THE ROSCOE B. JACKSON MEMORIAL LABORATORY

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With a Chapter on

INFECTIOUS DISEASES OF MICE

by

J. H. DINGLE

Harvard Medical School

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To the Trustees of

The John and Mary R. Markle Foundation

Whose generous grant

Made its preparation possible

This book is dedicated

PREFACE

Of all the laboratory mammals, probably none has contributed more to the advancement of knowledge than the common mouse. Certainly among all the mammals it is the most widely used, for not less than one million mice are raised each year in this country for research in bacteriology, cancer and genetics.

A result of this extensive use of the mouse is that a large body of information has grown up concerning it. This, however, is so widely scattered through the literature that it is often a major undertaking for the research worker who wishes to use it to locate and gather the particular facts that he needs. Much of this information is assembled in this book. In a number of cases, where there are important gaps in the literature, these have been filled in by special research projects. In general, controversial material has been avoided or given only brief mention. The emphasis is placed on established facts useful to the research worker.

Certain fields, for example anatomy and endocrinology, have of necessity been largely omitted. In most cases material omitted is adequately covered in other recent books.

Because it deals with the mouse alone, this book presents a vertical cross-section of biological knowledge rather than the more usual horizontal cross-section. It contains information about one animal drawn from various branches of zoology, rather than information about one branch of zoology drawn from observation of a variety of animals. There is, I believe, one notable virtue in this vertical method of presentation, namely, that it makes the synthesis of biological knowledge somewhat easier. There is a widespread feeling among biologists that progress will depend increasingly on the synthesis of the specialized techniques which have been developed within the individual cubby-holes into which science is somewhat arbitrarily divided. The departmentalization of biology is a convenience not to say an absolute necessity, but within the organism the tissues, the genes, the endocrines, the diseases and the processes of development are all intimately related, and the biologist frequently finds that research in his own specialty is leading him straight into another field of

knowledge. At the present time there are, for example, increasingly well beaten paths between genetics and embryology, between endocrinology and cancer research, between cancer research and bacteriology, between bacteriology and genetics. It is a major purpose of this book, by gathering together the fundamental knowledge about the mouse from several fields of study, to make it easier for the research worker using mice as his experimental material to traverse these interconnecting paths of science.

The preparation of the book has been financed by a grant from the John and Mary R. Markle Foundation. This generous support has made possible the conduct of several pertinent research projects and the preparation of many original photographs and drawings. The embryological studies described in Chapter 1 have also been aided by a grant from the Alexander Dallas Bache Fund of the National Academy of Sciences. In the preparation of their material the authors have been ably assisted by the following persons: Miss Olive Bartholomew, preparation of embryological and histological sections; Miss Bernette Bohen, drawings; Mr. Joshua Burnett, tabulation of linkage data; Dr. Elizabeth Chase, histological sections; Dr. Katrina P. Hummel, photography; Mr. Arthur Lieberman, bibliography; Mr. John Mowat, photography and construction of apparatus; Mr. William Payne, photography; Miss Ella Rowe, preparation of sections; Miss Elizabeth Keucher, assistance in preparation of the index. Prof. C. H. Danforth has made valuable suggestions in regard to several parts of the text.

In conclusion, the editor would like to express his appreciation to the other members of the Laboratory Staff for their continued cooperation and for many valuable suggestions, and to Dr. C. C. Little for his hearty support and, in a broader sense, for the wise direction in a large measure responsible for the friendly atmosphere so essential for successful collaboration.

GEORGE D. SNELL, *Editor*
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Chapter 1

THE EARLY EMBRYOLOGY OF THE MOUSE

By George D. Snell, *Roscoe B. Jackson Memorial Laboratory.*

The early embryology of the mouse and rat has been the subject of numerous studies during the past 50 or 60 years. Because the results of these studies are published in several languages and in many different journals, some of them not accessible in most libraries, because errors were inevitably present in the earlier articles, and because many of the published figures are not adequate for conveying a quick and clear understanding of the subject, the author has undertaken, and here presents the results of, a complete reinvestigation of nearly the whole field. The material used in the study consists of sections of embryos spaced at six hour intervals from 4 days to 9 days. In some cases ten or more embryos of a single stage have been sectioned. The sections were prepared by Olive Bartholomew, Elizabeth Fekete and the author. The technique used has been described elsewhere (14). To this description need only be added that, because in most cases the females used as mothers were hybrids between two strains, and because the fathers were from a third strain, thus giving both embryos and mothers a maximum of hybrid vigor, the stages as here described are usually earlier, often by as much as a day or more, than comparable stages described by other authors. While this procedure gave embryos which developed rapidly and were normal in a high proportion of all cases, it did not eliminate variability. No attempt has been made to describe the varia-

tions that have been noted in the rate of development of embryos or in the rate of development or form of separate parts. It should be emphasized, however, that the range of variation in these respects is considerable.

Wherever it is applicable to the mouse we have in general followed the terminology employed by Patten in the "Embryology of the Pig."

Contentious material is described in footnotes rather than in the text. Some readers will wish to skip these altogether. A complete bibliography is given at the end of the chapter, including a number of articles not referred to anywhere in the text.

Fertilization.—By fertilization is meant the entrance of a sperm into the egg. Fertilization in the mouse occurs in the upper end of each oviduct where the eggs are found, usually gathered into clumps, after their discharge from the ovaries. The sperm thus have to traverse the length of the uterus and oviduct to reach the eggs, a process accomplished partly through their own motility but for the most part through a churning action of the female duct. Since the beginning of heat in the female commonly occurs about two hours before ovulation, sperm may already be present in the oviduct when ovulation occurs.

The egg consists of a sphere of living protoplasm, the vitellus, surrounded by a transparent, non-living membrane, the zona pellucida (Fig. 1A). The zona pellucida in turn is surrounded by follicular cells which, however, are dispersed soon after fertilization. Within the vitellus is the egg nucleus, not clearly visible in living eggs such as the one shown in Fig. 1A, but easily seen in fixed and stained material.

Mature eggs within the ovary average about 95 μ in diameter (outside diameter of the zona). Following fertilization the zona pellucida expands until its outer diameter becomes about 113 μ (= .0044 inches). This is just within the limits of visibility for the unaided eye (35).

Usually only one sperm enters each egg. Almost immediately after entry, which may occur through any part of the egg's surface, the vitellus shrinks slightly in size and the zona pellucida expands, so that a space forms between them (35, 50). This is the perivitelline space. At this time only the first polar body has been formed. Within the next few hours the second maturation division occurs and the second polar body is budded off from the surface of the vitellus (Fig. 1B).

Not only the sperm head but also the middle piece and sometimes the whole tail enters the vitellus. The sperm head carries in one complete set of chromosomes from the male parent, while the middle piece contributes mitochondria from this parent. These latter are soon distributed through-

out the vitellus, and at the first cleavage division are divided more or less equally, along with the mitochondria already present in the egg, to the two

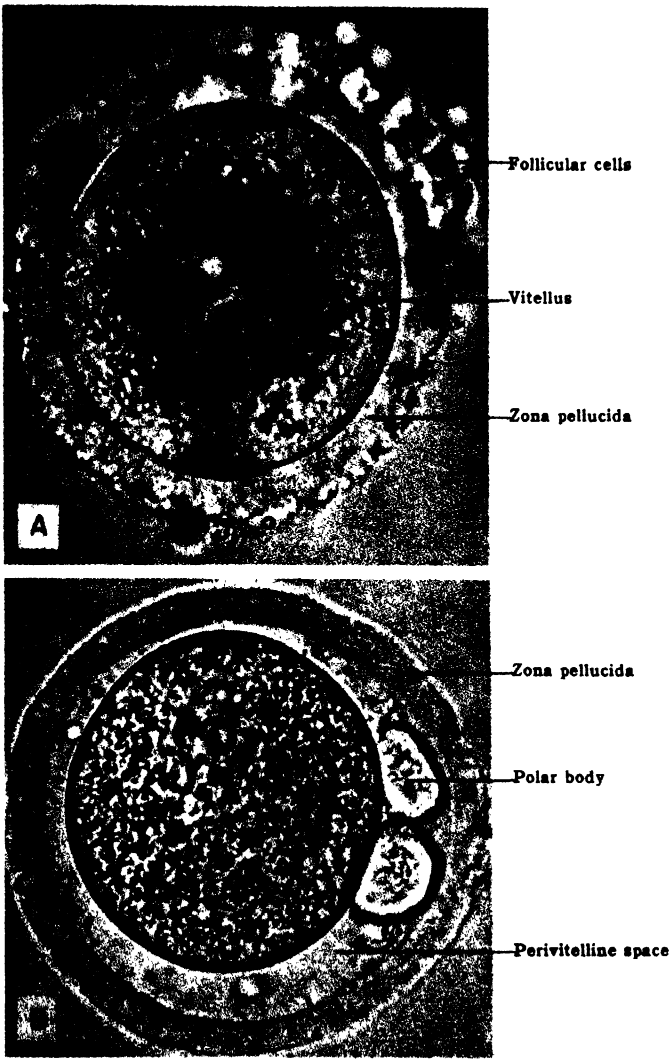


FIG. 1.—Photographs of mouse eggs (×600). A. Egg removed from ovary. B. Fertilized egg from oviduct 20 hours after copulation. Two polar bodies and sperm in perivitelline space. (*From Lewis and Wright.*)

daughter-cells. There is some evidence that Golgi material is also carried by the sperm into the egg (19, 22, 34).

The sperm and egg nuclei, now both within the vitellus, are known as the male and female pronuclei. They move towards each other until they lie

side by side, each appearing at this stage as a typical resting nucleus, though the male element is a little the smaller of the two. At the first cleavage division the nuclear walls break down, the chromosomes split longitudinally, and one-half of each split chromosome is carried to each daughter cell. Hence at this division, as at all future somatic divisions, each cell receives a full complement of chromosomes from each parent.

Cleavage.—Cleavage in the mouse occurs while the eggs are still in the oviduct. The first cleavage occurs about 24 hours after copulation and

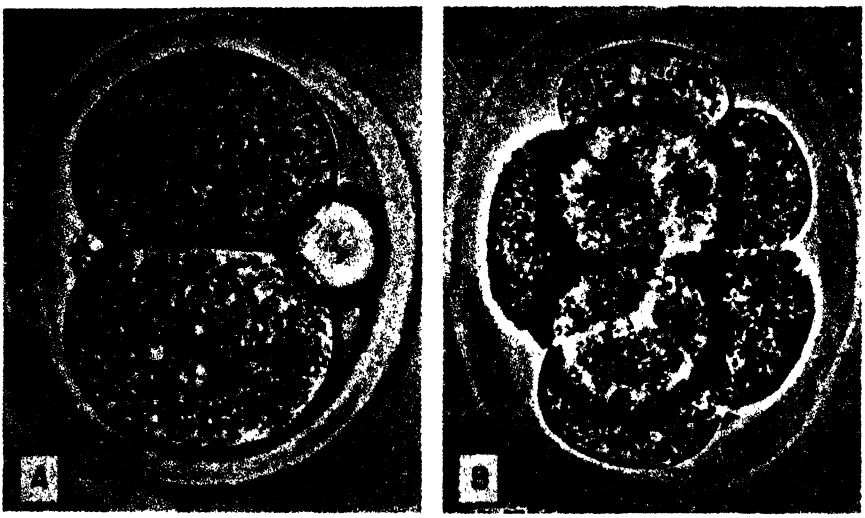


FIG. 2.—Photographs of mouse eggs (×600). A. Two-cell egg from oviduct 24 hours after copulation. Large second polar body and disintegrating first (on opposite side). B. Seven-cell egg from oviduct 48 hours after copulation. Note one cell on left larger than the rest. Division of this cell would give the eight-cell stage. (*From Lewis and Wright.*)

results in two cells not quite equal in size (Fig. 2A). Following divisions occur somewhat more rapidly, giving rise to 4-cell, 8-cell stages, etc., and are usually nearly synchronous in the different cells. Occasionally, however, eggs are found with some divisions completed, others still incomplete, and hence showing an odd number of cells (Fig. 2B). The actual act of division requires only 5 or 10 minutes; the interval between divisions lasts about 12 hours. Eggs of 16 cells or more, but in which no cavity has appeared, are called morulae. Eggs usually reach this stage about 60 hours after fertilization, and pass from the oviduct, through which they have been gradually moving, into the uterus, some 6 to 12 hours later (35). This is subject to

considerable variation, however, and in one study passage into the uterus at 4 days was found to be the rule (7).

The blastula.—Shortly after entering the uterus, and usually sometime after the egg has reached the 32-cell stage, an eccentrically located, fluid filled cavity appears among the cells of the morula. This enlarges rapidly

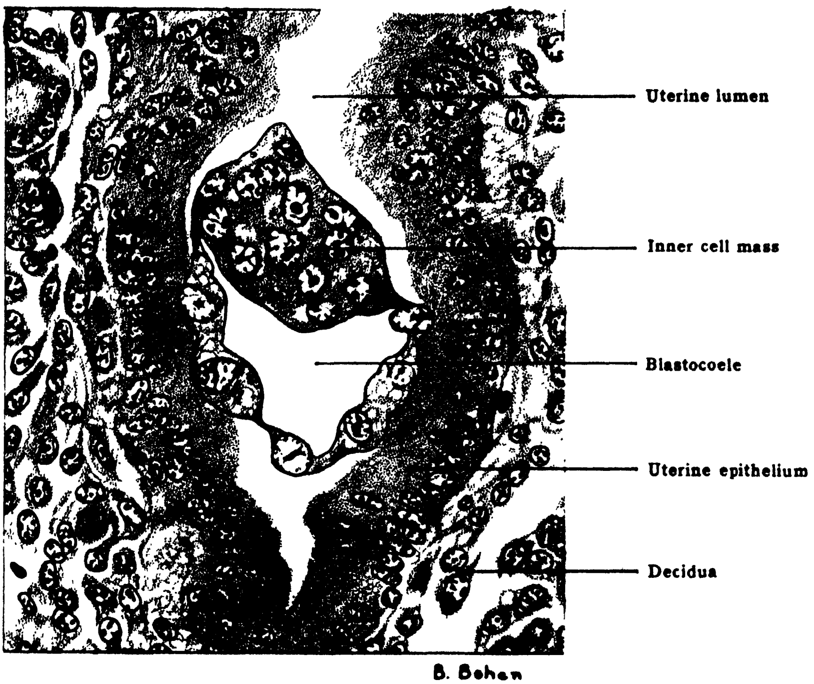


FIG. 3.—Blastula in uterine crypt 4 days after copulation. Projection drawing (×600).

to produce the segmentation cavity or blastocoele (Fig. 3). The cavity is bounded by only a single layer of cells except on one side where most of the cells are grouped to form a structure called the inner cell mass. Eggs in this stage are known as blastulae.

Implantation and early growth.—The uterus in the mouse is duplex, consisting of two horns which unite just anterior to their junction with the vagina, and each of which is attached to the dorsal body wall by a mesentery, the mesometrium (Fig. 4). There are two layers of muscle in each horn, an outer longitudinal layer and an inner circular layer. The uterine lumen is lined with epithelium. Between the epithelium and the muscle layers is the mucosa, a tissue which forms the bulk of the uterine wall. The epithelium is indented by numerous small crypts.

Very shortly after entering the uterus the eggs become spaced more or less evenly throughout its length, and each egg finds its way into a uterine

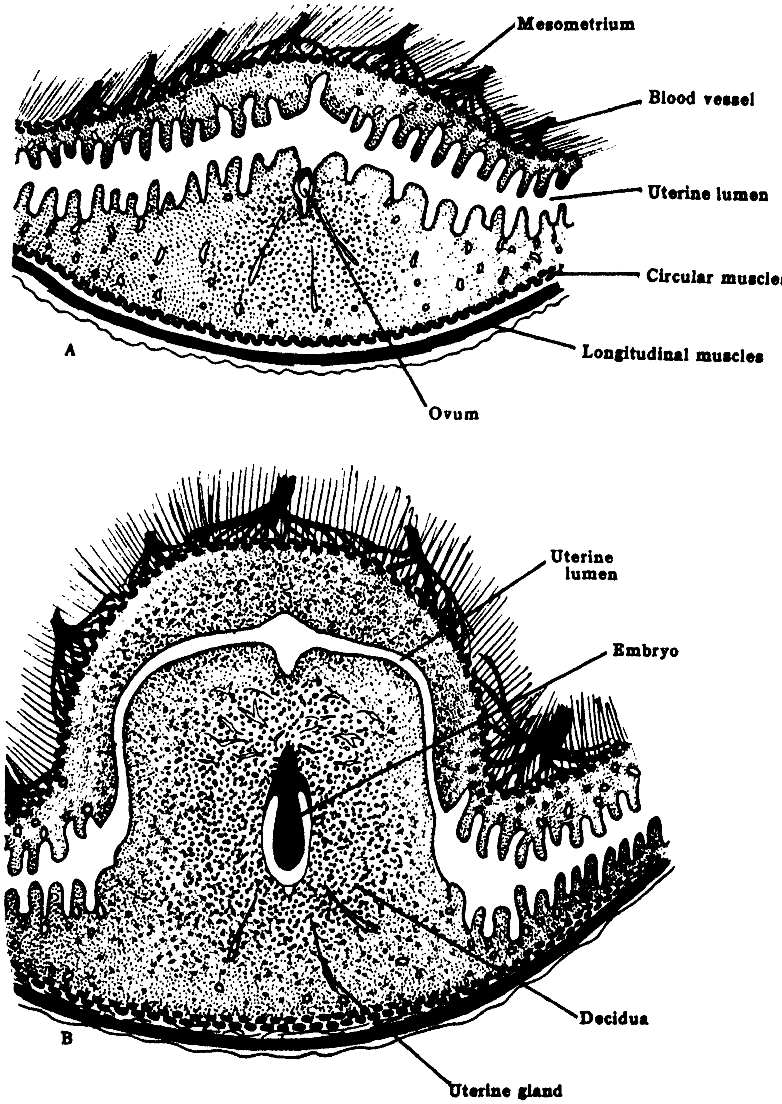


FIG. 4.—Diagrams showing implantation (×45). A. Longitudinal section through horn of uterus about 5 days after mating. An ovum has recently become implanted in one of the uterine crypts. B. Longitudinal section through implantation site about 7 days after mating. (*After Burckhard.*)

crypt on the ventral or antimesometrial side of the lumen, thereby coming into close contact with the uterine epithelium (Fig. 3). The presence of the

blastula quickly sets up changes at the implantation site. Within a few hours the epithelium begins to loosen, and its nuclei to show degenerative changes (Fig. 5). Within 15 hours it is sloughed off entirely (Fig. 6). At the same time active growth commences in the mucosa, so that by 1 day after implantation (5 days after mating) there is an appreciable swelling in the uterus at the implantation site. The swollen mucosa at the implantation site is known as decidua.

Meanwhile the zona pellucida has been lost from around the egg, perhaps through a process of digestion by means of an enzyme secreted by the uterine mucosa (11), though neither the exact time nor mechanism is thoroughly known.

Up to the time of implantation there has been no growth in size in the egg. Cleavage has resulted in a division of the egg, originally one large cell, into numerous smaller cells, but little if any new protoplasm has been formed in the process. Beginning with implantation, however, rapid growth commences. At first the blastocoele enlarges, while the inner cell mass assumes a flattened cup-shape with the concave face towards the cavity (Fig. 5). In the living condition the blastocoele is probably distended with fluid, and its walls tightly pressed against the uterine epithelium, but in fixed material at this stage there is always some collapse. This initial expansion of the blastocoele requires only a few hours and is quickly followed by a growth of the inner cell mass down into the enlarged cavity (Fig. 6). Blastocoele and inner cell mass both are known thereafter by new names; namely, yolk cavity for the former and egg cylinder for the latter. A comparison of Figs. 7, 8, 10 and 12 will show the rapid growth of the egg cylinder that occurs during the next two and one-half or three days.

The formation of the entoderm.—At the same time that the blastocoele begins to enlarge, the inner cell mass can be seen to be composed of two types of cells (Fig. 5). Adjacent to the blastocoele is a single layer of darkly staining cells. This is the entoderm, one of the three primary germ layers. The rest of the blastocyst is composed of ectoderm, divided into the ectoderm of the inner cell mass, and the trophectoderm, a single celled layer bounding the blastocoele ventrally and laterally. The trophectoderm (troph from the Greek word for nourishment) derives its name from the fact that it probably plays a rôle in the nourishment of the young embryo. The mesoderm has not yet appeared.

Very shortly after the first appearance of the entoderm, single cells or strands of cells grow out from its margin down along the inner surface of the trophectoderm. At first these cells are few and widely separated (Figs. 7

and 8), but by 6½ days they lie evenly spaced and quite close together over the trophectoderm's entire inner surface (Fig. 10). The layer of cells thus formed is known as the distal entoderm. Meantime the inner cell mass has grown down into the yolk cavity to form the egg cylinder. This is composed of an inner mass of ectoderm cells and an outer layer of entoderm cells (Fig. 8). This layer of entoderm cells bounding the egg cylinder is known as the proximal entoderm. The entoderm is thus divided into two distinct parts, distal and proximal, lining the distal and proximal walls of the yolk cavity.

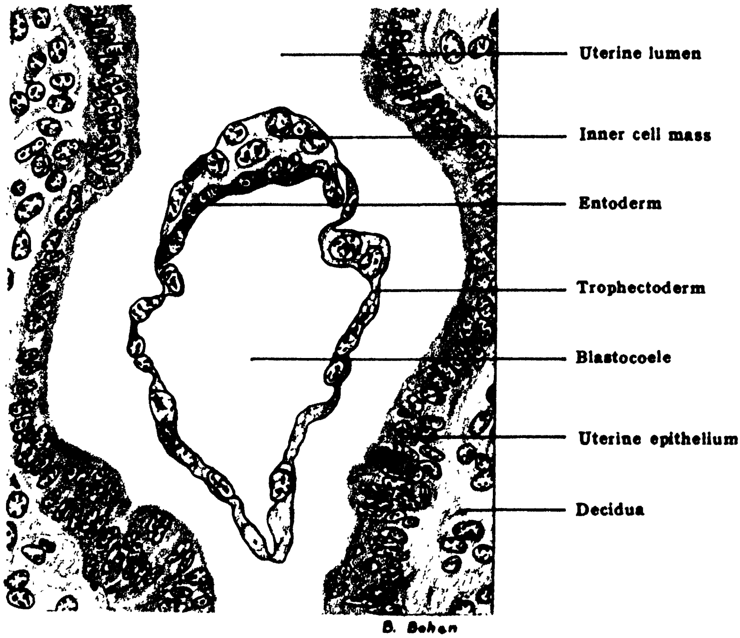


FIG. 5.—Section of implanting blastula 4 days 5 hours after mating. Projection drawing (×400).

Embryonic and extra-embryonic ectoderm.—At about 4½ days, when the egg cylinder first begins to form, it can be seen that the egg cylinder ectoderm is divided into two parts, a dorsal,* more darkly staining† region with

* Most authors have used the terms mesometrial and antimesometrial to distinguish the two poles of the egg, the former being toward, and the latter away from, the mesometrium or supporting mesentery of the uterus. However, as the dorso-ventral axis of the embryo coincides with the dorso-ventral axis of the mother for at least the first 8 days of development, the usage dorsal and ventral would seem to be perfectly clear in most cases besides having the advantage of simplicity. The dorsal side is up in the drawings.

† When counterstained with congo red.

elongated nuclei, and a ventral, more lightly staining portion with round nuclei* (Fig. 6). The former gives rise to various extra-embryonic struc-

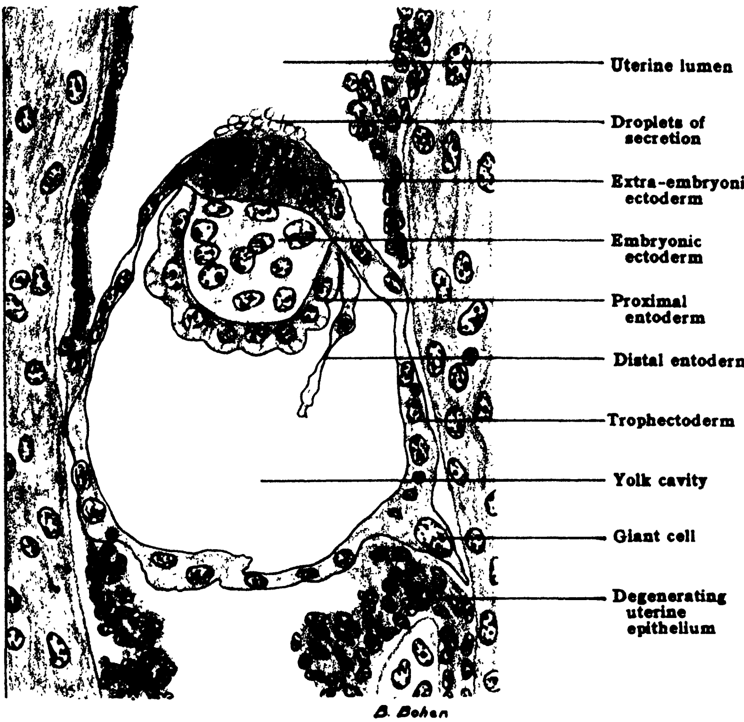


FIG. 6.—Longitudinal section of early egg cylinder stage at 4 days 15 hours after mating. Projection drawing (×400).

tures and is, therefore, called the extra-embryonic ectoderm; the latter gives rise to the ectoderm of the embryo proper and is, therefore, called the embryonic ectoderm. While the difference in staining reaction and in the shape of the nuclei has disappeared by 5½ days, the division between the

* It is possible that the division between embryonic and extra-embryonic ectoderm can be traced back to stages earlier than 4½ days. One author (41) contends that the ectoderm of the inner cell mass at a stage corresponding to that shown in Fig. 5 is divided into two regions, a lighter staining outer layer continuous with the trophectoderm and a darker staining area between this and the entoderm, but the existence of such a division has also been denied (22, 61). In our preparations at the 4¼ day stage we find occasional flattened, dark-staining nuclei on the outer surface of the inner cell mass and in some cases these appear to form a layer continuous with the trophectoderm. It seems probable that these represent an early stage of the extra-embryonic ectoderm. Phylogenetically the extra-embryonic ectoderm is probably derived from the trophectoderm, so that a similarity of structure is not surprising.

two regions is still quite distinct (Fig. 8). Strictly speaking the trophectoderm is also extra-embryonic ectoderm, but as a matter of convenience the term will be used only for the extra-embryonic ectoderm of the egg cylinder.

At about 5 days a cleft or cavity, the proamniotic cavity, appears in the embryonic ectoderm (Fig. 7). This is followed very shortly by the appearance of a similar cleft in the extra-embryonic ectoderm, and by the fusion of these two, so that by 5½ days the egg cylinder contains a narrow lumen (Fig. 8).

The ectoplacental cone.—Beginning at 5 or 5½ days, active growth at the dorsal end of the extra-embryonic ectoderm gives rise to a new structure,

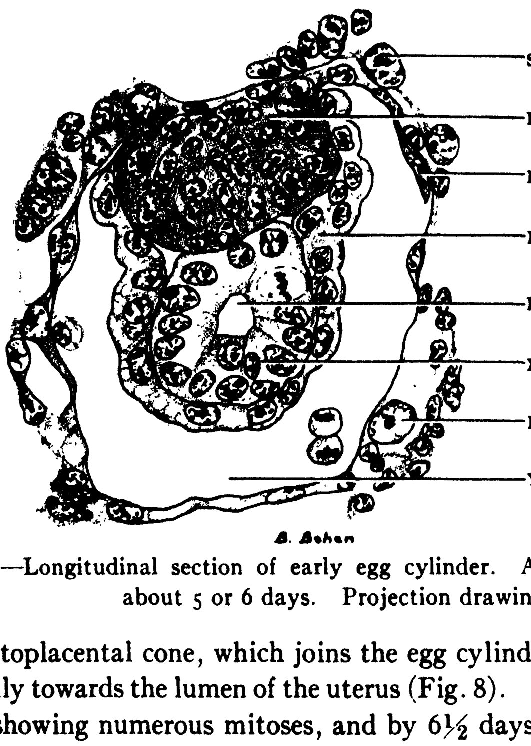


FIG. 7.—Longitudinal section of early egg cylinder. Age unknown, but probably about 5 or 6 days. Projection drawing (×400).

the ectoplacental cone, which joins the egg cylinder ventrally, and extends dorsally towards the lumen of the uterus (Fig. 8). This develops rapidly, its cells showing numerous mitoses, and by 6½ days it composes almost one-half of the total length of the embryo. Its structure, particularly at the upper extremity, is porous, and the interstices between the strands of cells that compose it soon become infiltrated with maternal blood (Fig. 10). In later stages it becomes part of the placenta.

The inversion of the germ layers.—At 5½ days (Fig. 8) the egg cylinder is a structure consisting of a double wall enclosing a narrow lumen. The inner layer of the double wall is composed of ectoderm, the outer of entoderm. This relation of ectoderm and entoderm, found in the mouse, rat,

rabbit, guinea pig and their close relatives, proved very puzzling to early embryologists, for the reason that it is the reverse of the condition found in

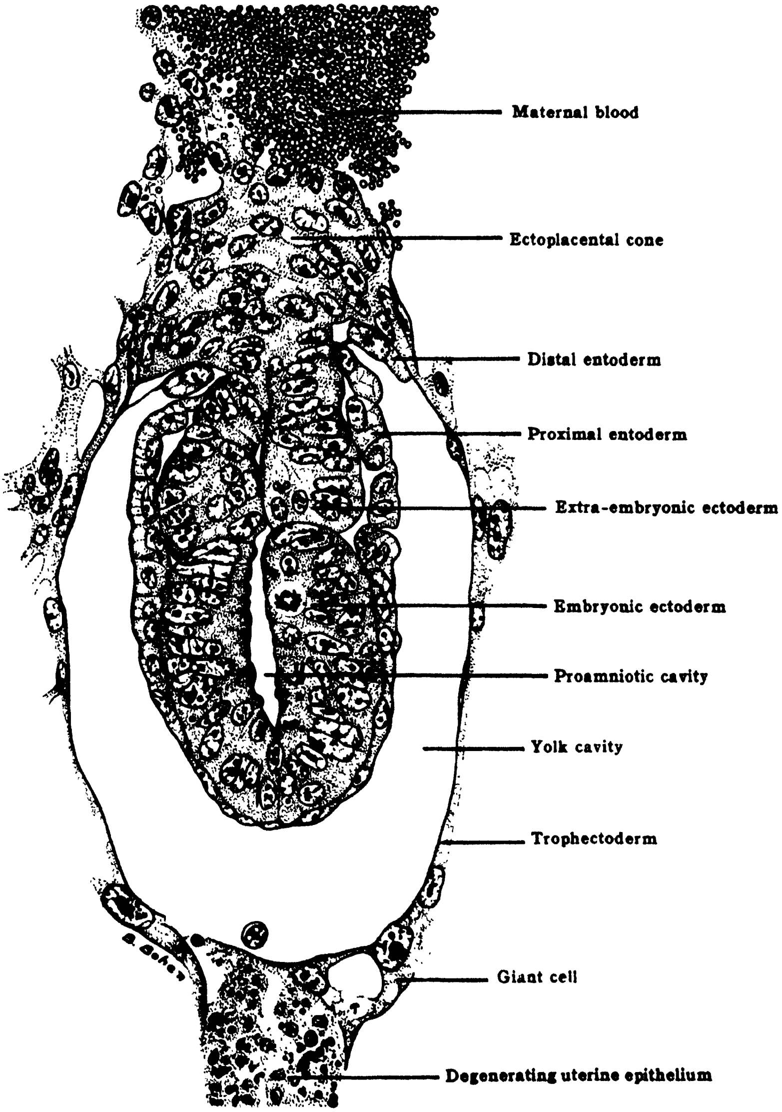


FIG. 8.—Longitudinal section at 5 days 12 hours after mating. Projection drawing (×300).

all other chordates. It has been called the inversion of the germ layers. While at first sight it seems to indicate a drastic alteration in early develop-

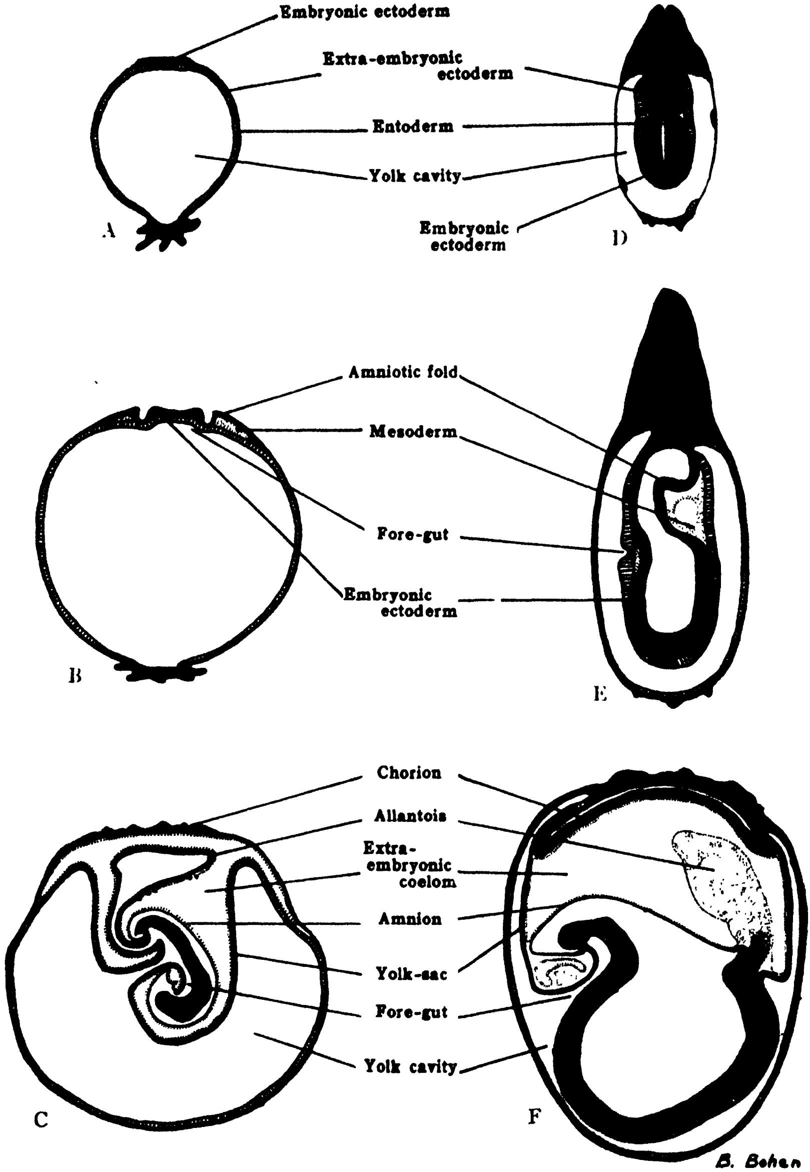


FIG. 9.—Diagram comparing the early stages of development in a primitive rodent, the thirteen-striped ground squirrel, and in the mouse. A, B and C. The thirteen-striped ground squirrel. (*After Mossman and Weisfeldt.*) D, E and F. The mouse (*Mus musculus*). (A and B, ×24; C, ×8; D and E, ×100; F, ×50.)

ment, actually there is no very fundamental change in the relations of the important structures. Those changes that are involved are easily understood from a comparison of the early development of the mouse with that of a primitive rodent, the thirteen-striped ground squirrel. Three comparable stages for each species are shown diagrammatically in Fig. 9. Beginning students of embryology will want to refer again to this figure after completing the study of later stages in the mouse.

In primitive rodents, as represented by the thirteen-striped ground squirrel, the embryonic area (embryonic ectoderm and underlying entoderm) forms a disc that overlies an almost spherical yolk cavity. In the mouse, the embryonic area forms a deep cup pushed far down into the yolk cavity, which thereby is greatly reduced in size. The obvious explanation of this condition is that during some period in the evolution of the mouse there developed an invagination of the embryonic area into the yolk cavity, the curvature of the embryonic area thereby being reversed and the relation of ectoderm and entoderm inverted. The change is comparable to that produced when a rubber ball has one side pushed in, being altered thereby from a sphere to a cup.

In Fig. 9D the lumen of the egg cylinder is shown extending through the ectoplacental cone to the outside. This condition is probably the exception rather than the rule, but it has been described by Sobotta (61) and Melissinos (41), and we have found it in a few cases in our material. It is significant evidence for the theory that the inversion of the germ layers is due to an invagination of the embryonic area.

Further evidence is provided by the later development of the thirteen-striped ground squirrel (Fig. 9C). In this species the whole embryo sinks down into the yolk cavity, carrying the splanchnopleure with it. The splanchnopleure is thereby inverted, but no inversion of embryonic ectoderm and entoderm occurs because of the advanced development of the embryo at the time. However, if the sinking or invagination of the embryonic area were pushed back to an earlier period of development, the condition found in the mouse would result.

One interesting consequence of the inversion of the germ layers is the production of a very compact form of early development. Much seemingly waste space in the yolk cavity is eliminated. The reader should note in this connection that the drawings of the mouse embryos in Fig. 9 are at a higher scale of magnification than those of the ground squirrel embryos. Actually, at comparable stages of early development, the total volume of a mouse embryo is, in round figures, perhaps one-fiftieth that of the total volume of

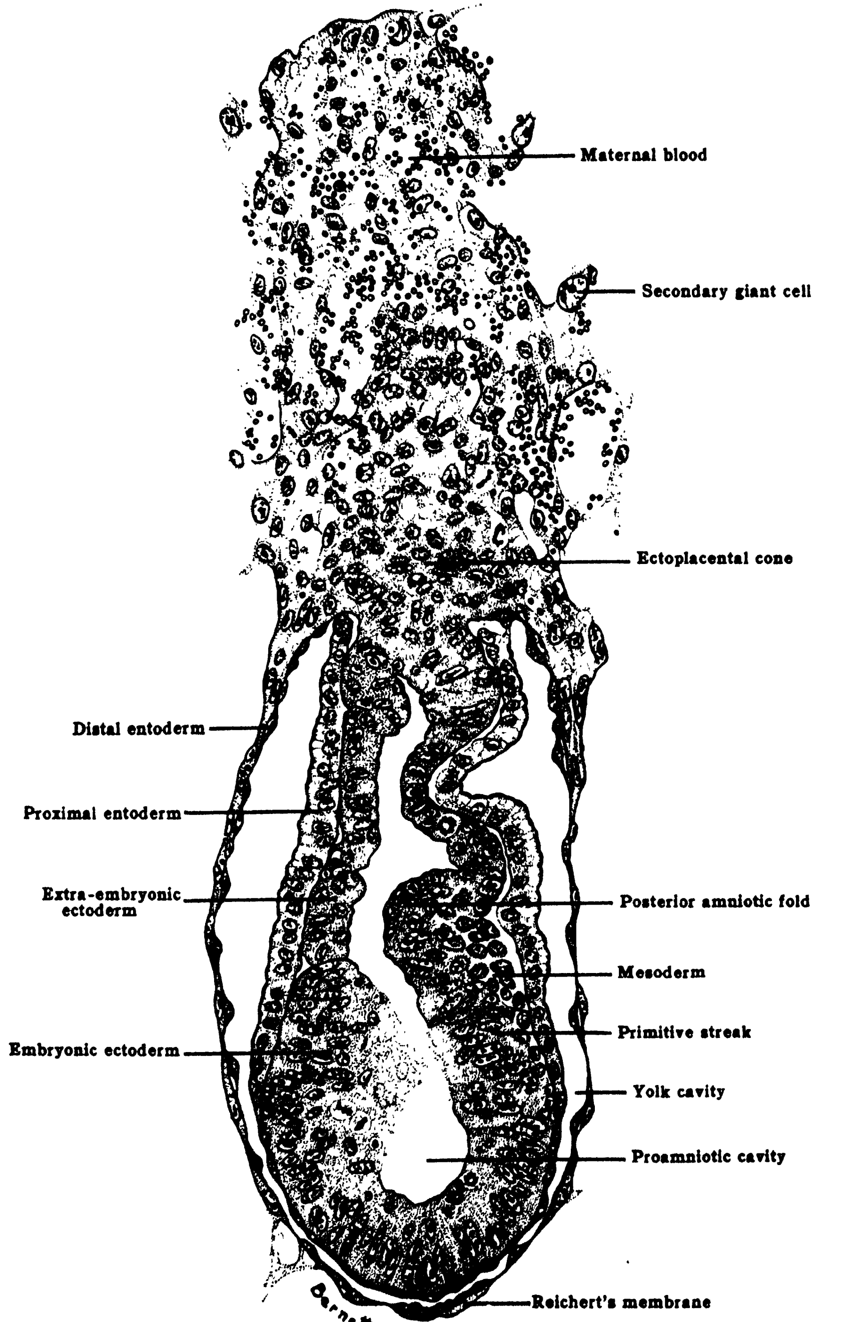


FIG. 10.—Sagittal section of mouse embryo of 6 days 13 hours, showing early stage of mesoderm formation. Projection drawing (×300) except that Reichert's membrane is drawn in part from adjacent section.

an embryo of the thirteen-striped ground squirrel. This reduction in total volume involves little if any reduction in the volume of the embryonic area proper.

The primitive streak and mesoderm formation.—At 6½ days the middle germ layer or mesoderm makes its appearance (Fig. 10). The first mesoderm cells are budded off from a narrow strip of embryonic ectoderm which extends dorso-ventrally from the line of junction of the embryonic and extra-embryonic ectoderm about half way to the tip of the egg cylinder. This strip of ectoderm is known as the primitive streak. Since the primitive streak lies at the posterior end of the embryo, an anterior-posterior axis is at once established with its appearance.*

The mesoderm cells form a loose tissue of very characteristic appearance. They multiply rapidly, wedging their way laterally between ectoderm and entoderm toward the anterior margin of the egg cylinder (Fig. 14A). The forward growth is particularly rapid along the line which marks the junction between embryonic and extra-embryonic ectoderm, and in this line mesoderm may be found at the anterior margin of the egg cylinder about 12 hours after the first mesoderm cells appeared (Fig. 12). Elsewhere the two lateral wings of mesoderm do not penetrate to the mid-sagittal region until much later. Some mesoderm cells also push dorsally between the extra-embryonic ectoderm and the adjacent entoderm, thus leaving the region of the embryo proper. These mesoderm cells, for the most part, are destined to take part in the formation of the yolk-sac, an extra-embryonic membrane, which later envelops the embryo and which is discarded at birth.

The orientation of the embryo in the uterus.—Since the primitive streak is at the posterior margin of the egg cylinder, its formation, heralded by the appearance of the mesoderm, establishes an anterior-posterior axis in the embryo. It is appropriate at this point to consider how this axis and the other axes of the embryo are oriented in relation to the uterus.

At the time of implantation the embryo settles to the ventral or anti-mesometrial side of the uterus. When it first implants, the inner cell mass is up or towards the mesometrium, the blastocoele is down or away from the

* In our material we have noted that from 5 to 5½ days, the egg cylinder and more particularly the proamniotic cavity instead of being round in cross section, are slightly flattened along an axis perpendicular to the mesometrium. This is the same as the future anterior-posterior axis. However, it cannot be determined until the appearance of the mesoderm which end of the axis is anterior and which end posterior. With the appearance of the mesoderm the flattening of the egg cylinder, if any, is along the opposite axis.

mesometrium (Fig. 4A). In terms of an older embryo, the ectoplacental cone is up and the embryonic portion of the egg cylinder is down (Fig. 4B). The dorso-ventral axis of the embryo is thus parallel to the mesometrium and perpendicular to the long axis of the uterus (Fig. 11). The anterior-posterior axis of the embryo likewise has a definite orientation with respect to the uterus, being, as a rule, perpendicular to the mesometrium. Depar-

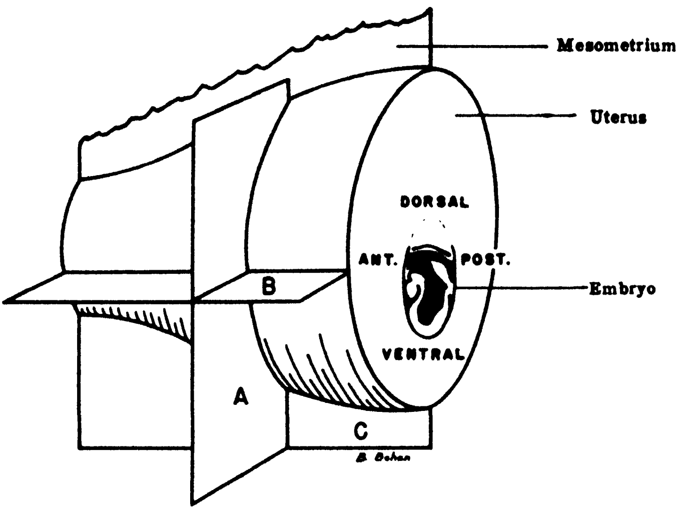


FIG. 11.—Diagram showing the orientation of an 8 day embryo in the uterus, and of the planes in which sections are cut. Plane A: transverse to uterus, sagittal to embryo. In the early egg cylinder stages this may be referred to also as a longitudinal section of the egg cylinder. The orientation of the embryo is not always consistent and may sometimes depart by as much as 45° from this plane. Plane B: transverse section of embryo. Note, however, that in embryos past the egg cylinder stage this plane though transverse to head and tail regions is frontal with respect to the mid-trunk region. Plane C: frontal section of embryo. Note, however, that in embryos past the egg cylinder stage this plane though frontal to head and tail regions is transverse with respect to the mid-trunk region. In early egg cylinder stages this may be referred to also as a longitudinal section of the egg cylinder.

tures from this orientation by as much as 45° may, however, occur. This orientation persists until about 8 or 8½ days when the embryo begins to shift its position in the uterus.

Amnion, chorion and exocoelom.—When mesoderm cells first appear between the ectoderm and entoderm at the posterior margin of the egg cylinder, they cause the ectoderm at the line of junction between its embryonic and extra-embryonic portions to bulge into the proamniotic cavity.

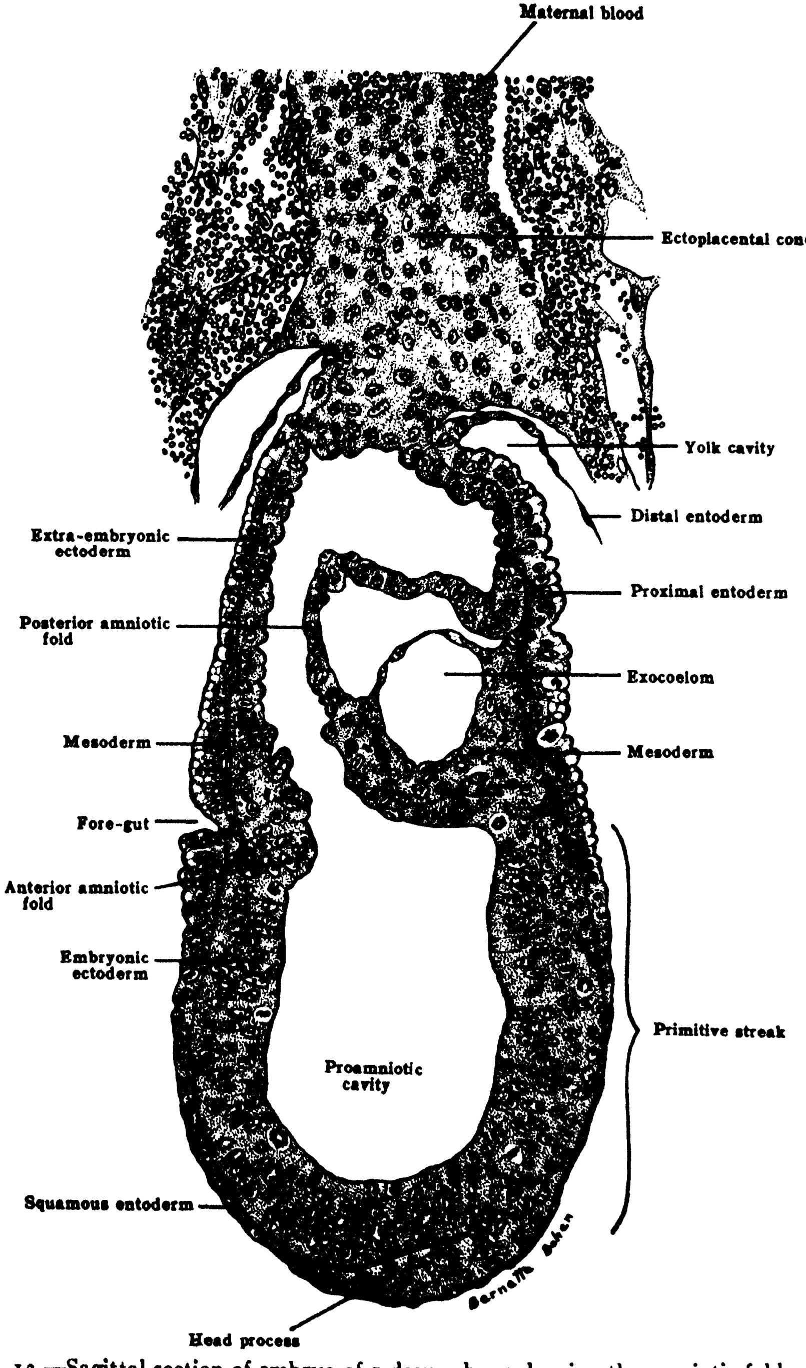


FIG. 12.—Sagittal section of embryo of 7 days 1 hour showing the amniotic folds and the head process. Reichert's membrane omitted. Projection drawing (×300).

This bulge is the beginning of the posterior amniotic fold* (Fig. 10). In like manner the lateral wings of mesoderm, progressing around the egg cylinder toward its anterior margin, give rise to folds along the sides of the cylinder. These are the lateral amniotic folds. Finally, when the mesoderm reaches the anterior margin of the egg cylinder, a small anterior fold is produced (Fig. 12). The posterior, lateral and anterior folds should be thought of, not as separate structures, but as a continuous constriction about the middle of the egg cylinder which is drawn tighter and tighter as the folds develop. Because of the very precocious development of the posterior amniotic fold as compared with the anterior one, the constriction is eccentric, the point of final closure being far towards the anterior margin of the egg cylinder.†

Before the anterior fold forms, small cavities‡ begin to appear in the mesoderm of the posterior and lateral folds. These soon coalesce to form a single large cavity, the extra-embryonic coelom, or, more concisely, the exocoelom (Fig. 12). The exocoelom at this stage, and at all future stages, is lined by mesoderm. For a short time a second cavity is present in the posterior amniotic fold between the mesoderm and ectoderm (Fig. 12), but this is a transitory structure of no particular significance.

In less than a day after the first appearance of the amniotic folds, the girdle which they form has closed. For a time a vertical strand of cells adjacent and usually attached to the inner anterior wall of the exocoelom marks the point of closure, but this soon disappears and the separation is complete. The resulting condition is shown in the sagittal section reproduced in Fig. 13.

Three cavities§ are now present in the egg cylinder in place of the single proamniotic cavity which it formerly contained. The most ventral of these

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* In Fig. 10 there may be seen a second fold pushing into the proamniotic cavity just dorsal to the posterior amniotic fold. Sobotta (62) shows this in his Fig. 5, but interprets it as an artifact. Our material would indicate that it is regularly though briefly present. Its significance is unknown, but it is perhaps indicative of the very rapid growth that occurs in the whole posterior wall of the egg cylinder at the time of mesoderm formation.

† In the rat, the anterior amniotic fold is much better developed than in the mouse, and the constriction, therefore, less eccentric (26).

‡ None of the embryos in our collection show this early stage in the formation of the exocoelom. This description is based on the observations of Jolly and Férester-Tadié (26).

§ In some cases, also, a transitory fourth cavity, the cavity between ectoderm and mesoderm mentioned on page 18, second paragraph, is present.

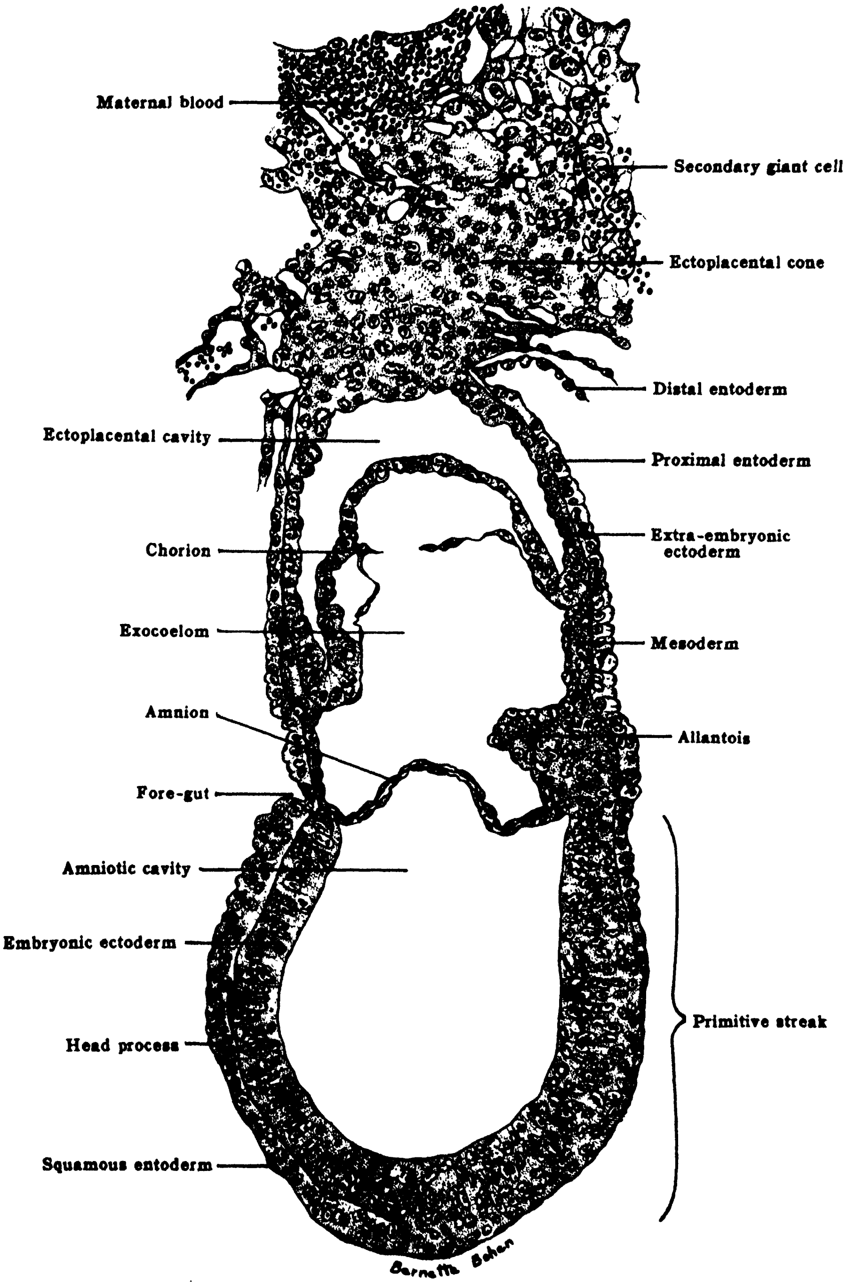


FIG. 13.—Sagittal section of embryo of 7 days 6 hours showing completion of amnion formation. Reichert's membrane omitted. Projection drawing (×200).

is the amniotic cavity, lined with embryonic ectoderm.* At this stage it is cup-shaped, as can be seen from Figs. 13 and 14A, which show it in sagittal and cross section respectively.

In the middle is the exocoelom, lined with mesoderm.

At the dorsal extremity of the egg cylinder is the ectoplacental cavity, lined with extra-embryonic ectoderm. The amniotic cavity and the exocoelom, though neither one, as will be seen later, is included in the actual body of the embryo, are important in its future development. The ectoplacental cavity, on the other hand, already the smallest of the three, gradually becomes narrower and finally disappears.

The membrane separating the amniotic cavity from the exocoelom is called the amnion. It is composed of two thin, cellular layers, one of ectoderm, the other of mesoderm. Separating the exocoelom from the ectoplacental cavity is another membrane, the chorion, likewise composed of ectoderm and mesoderm.

The head process.†—It will be remembered that mesoderm is first proliferated by the primitive streak in embryos about 6½ days old. The growth is entirely from the lateral and caudal margins of the primitive streak; no mesoderm is proliferated from its cephalad extremity. Beginning at about 7 days, however, growth does occur in this region, but the structure formed shows greater affinity to the entoderm than to the mesoderm. It is known as the head process. In sagittal sections it first appears as a wedge shaped group of cells between the ectoderm and entoderm at the ventral extremity of the egg cylinder (Fig. 12). The base of the wedge is attached to the ventral end of the primitive streak from which it takes its origin; the tip of the wedge points forward towards the anterior margin of the egg cylinder. Cells grow out rapidly from the margins of the wedge, forming a thin, spreading sheet between ectoderm and entoderm.‡

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* We interpret the ectoderm of the amnion as embryonic ectoderm, the ectoderm of the chorion as extra-embryonic ectoderm. The evidence on this point is not necessarily conclusive, however, for concurrent with the appearance of the amniotic folds, the division between the two types of ectoderm loses much of its distinctness.

† Sobotta (62) studied embryos representing the stages during which the head process develops, but his drawings indicate that his otherwise admirable sections were not close enough to the exact sagittal plane to reveal this structure clearly. Consequently, it remained for Jolly and Férester-Tadié (26) to first describe it correctly for the mouse and rat. Our observations are entirely in accord with theirs.

‡ The entoderm and the margins of the head process are so thin and close together at this stage that favorable conditions are necessary to distinguish them. In the

If the reader now will refer back to Fig. 10, he will see that the entoderm over the ventral extremity of the egg cylinder is stretched and the cells flattened, but that near the upper margin of the embryonic portion of the cylinder there is a sudden change to a higher type of cell. The transition is particularly abrupt at the anterior margin of the cylinder. The thin or flattened entoderm we shall refer to as squamous entoderm, the thick entoderm as columnar entoderm, the line of junction between the two as the transition line. The reader should take time at this point to note, in Figs. 10 and 12, the precise location of the transition line.

The limits of the head process are as follows. Caudad, it begins at the anterior extremity of the primitive streak, that is to say just a little above and caudad to the ventral tip of the egg cylinder. Cephalad, it extends to the transition line. Laterad, at its broadest point it may extend almost around the anterior half of the circumference of the egg cylinder (Fig. 14A), but mostly it is narrower than this, filling perhaps the anterior fifth of the egg cylinder's circumference.

When its forward growth brings it to the transition line, the head process fuses with the columnar entoderm with which it has thus newly come in contact (Fig. 13). The fusion is so complete that in later stages the line of junction is completely lost. Laterally, its outer margins fuse with the squamous entoderm. Meantime the squamous entoderm underlying the head process, already very thin, becomes increasingly attenuated, its nuclei become widely separated and very flat, and the cytoplasm largely disappears (Figs. 14A and B). At 7½ days no further trace of it remains.

In the course of the upward and laterad growth of the head process and the forward growth of the mesoderm the two cell layers come in contact and overlap (Fig. 14A). In the regions of overlapping, the head process stays adjacent to and advances over the surface of the entoderm, while the mesoderm remains next to the ectoderm. At 7½ days the development of the mesoderm has brought it between ectoderm and head process everywhere except for a strip along the mid-sagittal plane of the embryo. As we shall see later, the head process of this mid-sagittal strip gives rise to notochord, while the remainder of the head process contributes to the lining of the gut.

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section shown in Fig. 12 there are several cells at the anterior limit of head process growth that cannot be classified definitely as either head process or entoderm. The division in the drawing in this region is partly arbitrary. When head process and mesoderm come into contact there is also possibility for confusion. However, in well fixed preparations cut at a favorable angle, the division in this case can almost always be precisely determined.

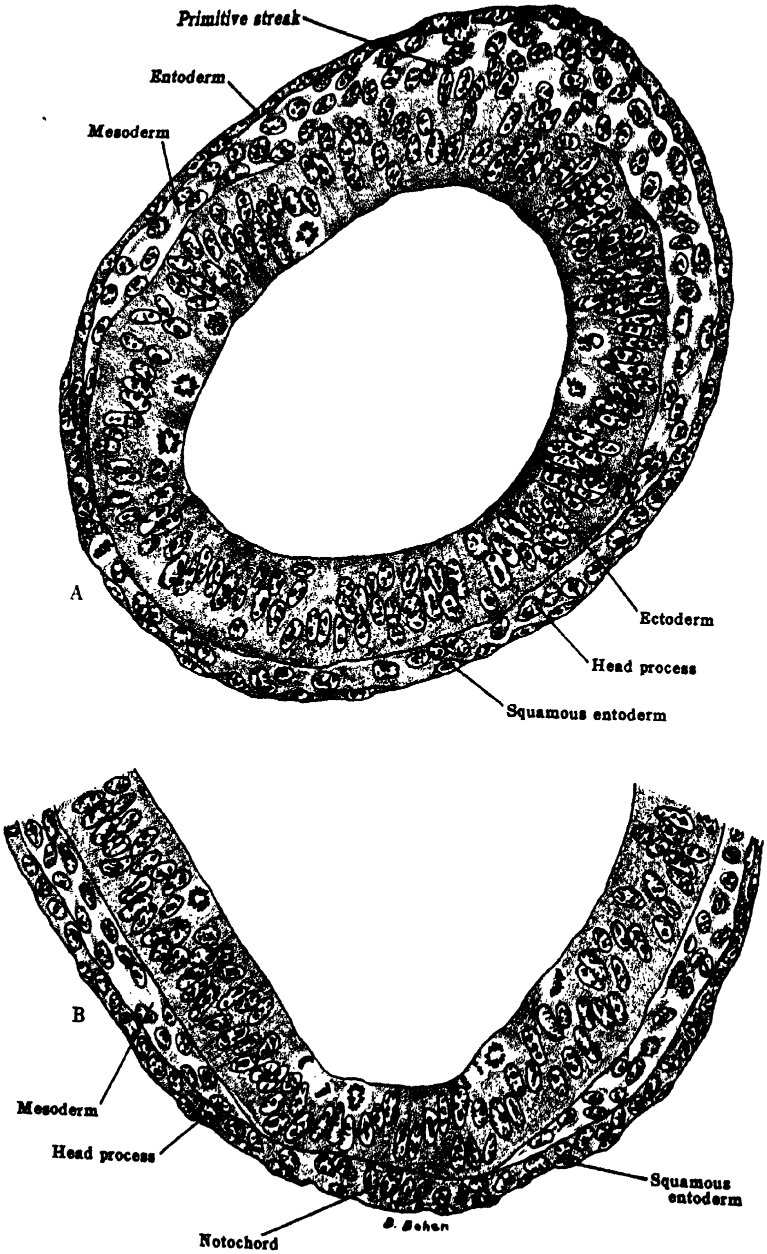


FIG. 14.—For descriptive legend see opposite page.

The neural groove.—It can be seen from Fig. 14B, which is a cross section of the anterior part of an egg cylinder of a 7¼ day embryo, that the ectoderm in the mid-sagittal plane forms a definite trough or truncated V. This

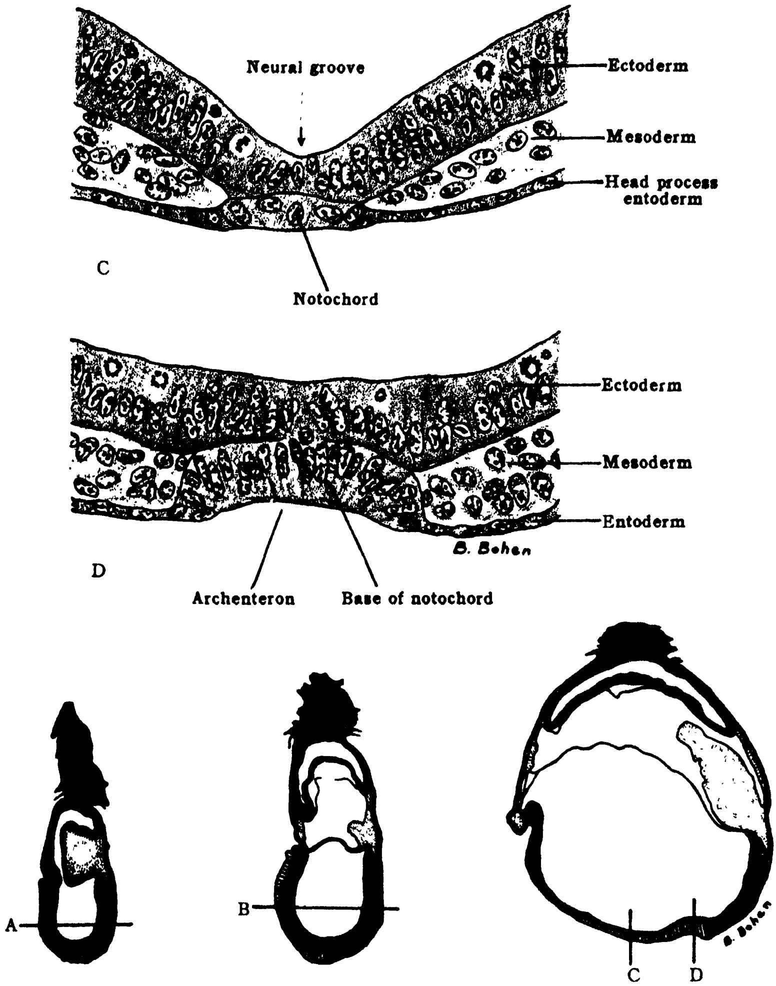


FIG. 14.—Transverse sections of head process. The location of sections is indicated on the small key diagrams. A. 7 day 1 hour embryo. B. 7 day 6 hour embryo. C and D. 7 day 10 hour embryo. Projection drawings (×400).

trough extends forward in the mid-sagittal plane from the cephalad end of the primitive streak well towards the cephalad limit of the embryonic ectoderm. Developed between the 7 and the 7¼ day stages (Figs. 14A and B), it is the beginning of the neural groove which later gives rise to the central nervous system. The further development of the neural groove will

be discussed later, but the reader will do well at this stage to look ahead to Figs. 19 and 20 which show the way in which it deepens and narrows and finally closes at the top to form the neural tube. The point to be emphasized here is that the appearance of the neural groove establishes a perfectly clear caudal-cephalic axis throughout the length of the embryo. The neural groove anteriorly and the primitive streak posteriorly lie in the precise mid-plane and together separate the right and left halves of the embryo.

The notochord.—At the same time that the neural groove is differentiating in the mid-sagittal area of the ectoderm, changes are also going on in the mid-sagittal region of the head process which immediately underlies it (Fig. 14). In this region the head process thickens, and the oval nuclei become oriented in general perpendicular to the ectoderm. Elsewhere it forms a thin membrane with the nuclei oriented parallel to the plane of the membrane. The structure thus differentiated ventral to and in contact with the ectoderm of the neural groove is the notochord. It is the axis about which the vertebral column is later laid down. The remainder of the head process, together with a part of the entoderm to which it is fused, becomes the lining of the gut.* This part of the head process will hereafter be referred to as gut entoderm. For a considerable period notochord and gut entoderm remain joined. Eventually, however, the two halves of the gut entoderm grow across the ventral surface of the notochord and unite in the mid-ventral line, leaving the notochord as an axial, rod-like structure between ectoderm and entoderm.

Huber (23) describes the head process in the guinea pig as giving rise to notochord only. Our material, however, confirms completely the contention of Jolly and Férester-Tadié (26) that in the mouse at least some gut entoderm is also derived from the head process. The critical stage is that shown in Fig. 14B in which it can be seen that the head process extends laterally considerably beyond the limits of the differentiating notochord.

A much mooted question is whether the notochord should be classed as ectoderm, entoderm or mesoderm (31). Since it is formed from the head process and since the very complete fusion of the margins of the head process with the entoderm indicate a close affinity between the two tissues, classification as entoderm would seem logical. If, however, head process is classed as entoderm, it must be remembered that its origin in time is quite different from that of all the other entoderm, and two separate stages of

* It seems likely that most or all of the mid-gut is lined by head process. Whether or not any of it enters into the formation of the fore- and hind-guts is not clear.

entodermal proliferation must be recognized. As to the place of origin, there is a certain similarity between the two tissues, one forming at the ventral margin of the inner cell mass, the other near the ventral tip of the egg cylinder, which is, so to speak, simply the inner cell mass grown up. Cell lineage studies might reveal a closer similarity in origin than is superficially apparent.

The archenteron.—At 7½ days there is a broad depression in the rather thick base of the notochord adjacent to its junction with the primitive streak (Figs. 14D and 15). The depression is a conspicuous landmark at this stage, but it is a transitory structure, the first signs of it appearing at 7¼ days and disappearance being complete about twelve hours later. It plays no part in later development and probably is best interpreted as a vestigial structure corresponding to a similar structure that occurs in more marked form in the development of reptiles,* and which in turn can probably be traced back to the archenteron of the lower chordates. On the basis of this probable homology it may be called the archenteron.

The allantois.—Soon after the exocoelomic cavity becomes well established, a process begins to grow out into this cavity from the mesoderm at the caudal end of the primitive streak. This is the allantois (Fig. 13), an extra-embryonic, mesodermal structure whose function is to convey blood vessels from the embryo to the placenta where they establish contact with the maternal circulation. In many vertebrates the allantois contains a cavity lined with entoderm and connected with the gut. There is no entoderm-lined cavity in mice; on the other hand there are numerous small cavities in the mesoderm giving the organ a porous structure.

After its first appearance at 7¼ days the allantois grows rapidly across the exocoelom in the direction of the ectoplacental cone (Figs. 15 and 16). Meantime the chorion becomes flattened against the base of the cone, constricting the ectoplacental cavity and finally eliminating it altogether. When the allantois makes contact with the chorion at about 8 days, a con-

* See for example Figs. 21 and 22 of Prentiss and Arey (51). We have found no trace of a neurenteric canal in the mouse, in the sense of a canal passing through the ectoderm and the base of the notochord and connecting amniotic cavity and yolk cavity. However, we have seen in a 7¼ day embryo a very short canal confined to the base of the notochord. The ventral wall was thin, and it may be presumed that it would shortly disappear, giving rise to the depression described above. Sobotta's Fig. 14 (62) shows a canal somewhat similar to the one we have noted, except that our material does not suggest, as his drawing does, that the canal is formed by invagination of the entoderm. Jolly and Férester-Tadié (26) have figured a section almost identical with ours.

tinuous structure is established connecting the posterior end of the primitive streak with the ectoplacental cone. In due course embryonic blood vessels

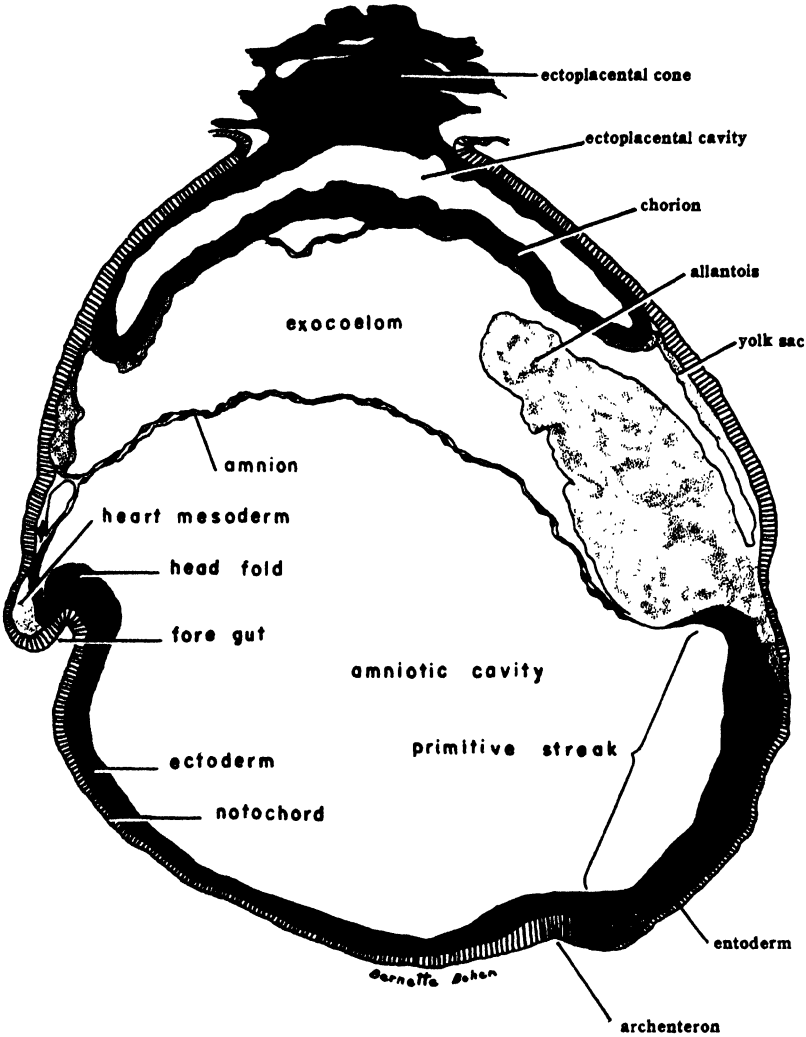


FIG. 15.—Sagittal section of mouse embryo of 7 days 15 hours. Reichert's membrane omitted. Projection drawing (×150).

will find their way along this pathway to make contact with the maternal blood supply.

Fore-gut and hind-gut.—In the early stages of its formation the digestive tract consists of three quite distinct parts, the fore-gut, the hind-gut and the mid-gut. These appear in the order named. The fore-gut can be traced

back to the 7 day stage when it consists merely of a deep notch in the thick or columnar entoderm at the anterior margin of the egg cylinder (Fig. 12). Six hours later there is little change (Fig. 13), but by 7½ days (Fig. 15) the notch has been replaced by a definite pocket in the entoderm, and the entoderm surrounding the pocket together with the overlying ectoderm form a bulge which projects into the amniotic cavity. From this stage on,

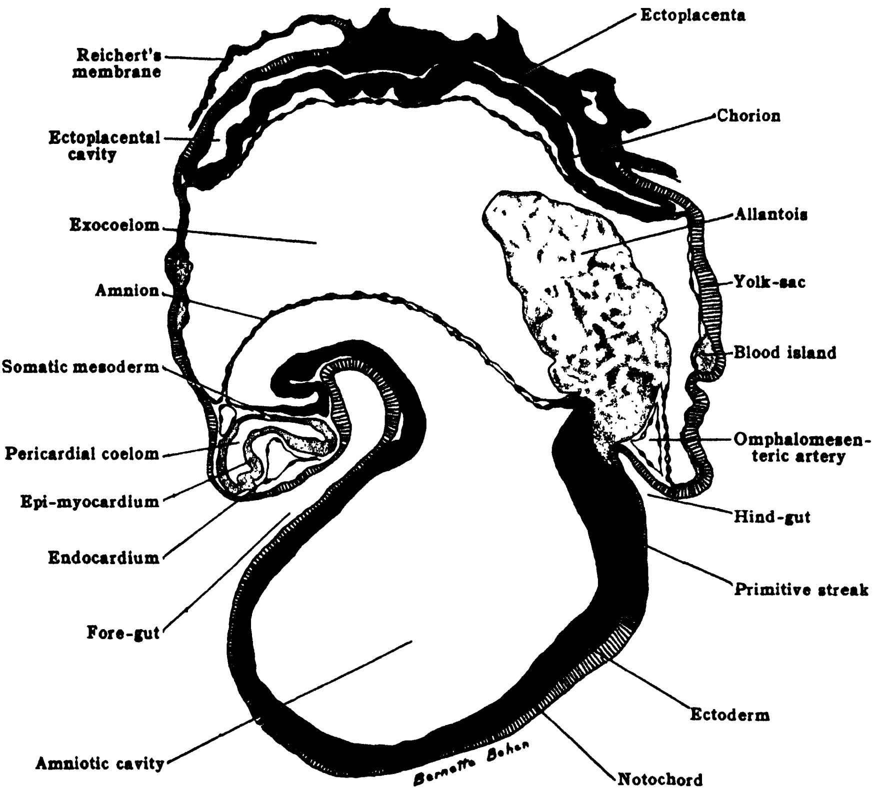


FIG. 16.—Partly diagrammatic sagittal section of embryo of 7 days 18 hours (×100). Reichert's membrane omitted.

growth of the fore-gut is exceedingly rapid, the pocket changing in a few hours into a deep pouch (Fig. 16). The process is due to a progressive drawing together in the mid-ventral line of the folds of entoderm that bound the anterior intestinal portal (Figs. 25C and 30), the portal thus being shifted farther and farther towards the cudal extremity of the embryo. The process has been aptly described as a "zipper action."

It should be noted that the fore-gut is lined by entoderm, and that this is surrounded by mesoderm and ectoderm. Thus in this region the process of

invagination has reversed the inversion of the germ layers found in the early egg cylinder. This is the first of the steps by which the germ layers in mice are brought into the relation characteristic of the adult, i.e., entoderm on the inside, ectoderm on the outside, mesoderm in between.

The hind-gut, less precocious than the fore-gut, appears at about 7¾ days as an invagination in the entoderm and overlying layers at the posterior end of the primitive streak (Fig. 16).

The open ends of the fore- and hind-guts are eventually joined by the mid-gut whose formation will be described in a later section. It is not these open ends, but the blind ends of the two guts which, by breaking through to the outside, give rise to mouth and anus. An early stage in the development of the mouth may be seen in 8 day embryos (Fig. 22). In the ectoderm of the head there is an invagination directed towards the anterior extremity of the fore-gut. This is the stomodaeum. The wall between the stomodaeum and the fore-gut is the oral plate. In course of time this ruptures and the mouth opening is thereby established. The anus develops in a similar manner at a somewhat later stage.

The head fold.—The invagination of the fore-gut involves a pushing or folding of the adjacent tissues into the amniotic cavity. The structure thus produced is the head fold (Fig. 15). First appearing at about 7½ days, it becomes a large and conspicuous structure within less than twenty-four hours (Figs. 22 and 26A). The growth of the neural folds in this region is more rapid than elsewhere, presaging the formation of the brain, and the heart, just ventral to the head fold, is also conspicuous by its rapid growth. In 8 or 8½ day embryos the difference in size between the head region and the middle of the trunk is striking. The head fold is thus the center of a region of particularly rapid growth (24).

The somites.—Since the somites are mesodermal structures, it will be useful before discussing their development to review the distribution of the mesoderm at the 7½ day stage when they make their first appearance. In the extra-embryonic region, the entire exocoelom is lined with mesoderm. The exocoelom contains also the allantois, a wholly mesodermal structure.* In the embryo proper there is little mesoderm in the mid-sagittal region. One small mass which will later contribute to the formation of the heart

* Not in the exocoelom, but also not part of the embryonic mesoderm, is a small mass of mesoderm at the anterior extremity of the amnion (Fig. 15). This is characteristically in the form of two hollow, thin-walled vesicles, one on each side of the mid-line, though the range of variation is considerable. That the vesicles are paired is probably due to the fact that the mesoderm in this region grew in from the two sides. Between the two vesicles, and hence approximately in the mid-line (it may be a little to one side or the other), is a very small area where ectoderm and entoderm are unsepa-

occurs anterior to the fore-gut (Fig. 15). The primitive streak in the mid-sagittal plane consists of a tissue which joins, and in structure is intermediate between, ectoderm and mesoderm. Whether or not this should be called mesoderm is a matter of definition. At the caudal extremity of the embryo its structure is essentially that of true mesoderm, and it may accurately be said that there is mesoderm in the mid-sagittal plane in this region.

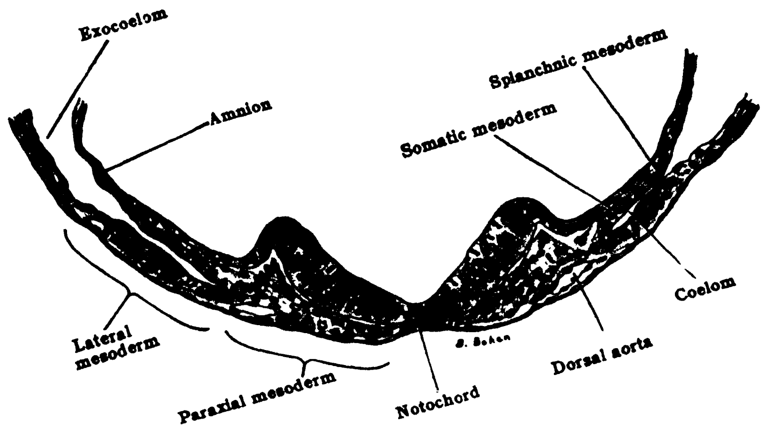


FIG. 17.—Section transverse to mid-trunk region of 8 day, 4 somite embryo. Through 2nd somite. Projection drawing (×150).

While the notochord blocks the entrance of mesoderm into most of the mid-sagittal area, there are well developed sheets of mesoderm on each side of this area. These lie between ectoderm and entoderm and are continuous laterally with the extra-embryonic mesoderm (Fig. 17). Anterior to the primitive streak, it is convenient to recognize two distinct areas in these mesodermal sheets, an area of paraxial mesoderm adjacent to the notochord, and an area of lateral mesoderm adjacent to the extra-embryonic coelom. The former gives rise to the somites, the latter to the mesoderm of the embryonic coelom. At 7½ days there is no visible division between the two areas (Fig. 19B), but beginning at about 7¾ days, coincident with the development of the somites, they are separated by a longitudinal cleft that becomes increasingly pronounced as the differentiation of the somites progresses (Fig. 17).

The somites are paired, segmental structures arising in the paraxial mesoderm (Figs. 18 and 25D). They are the first indication of metamerism in the developing embryo. The first pair forms a little anterior to the

rated by any mesoderm. This area is probably homologous with the proamnion of the rabbit.

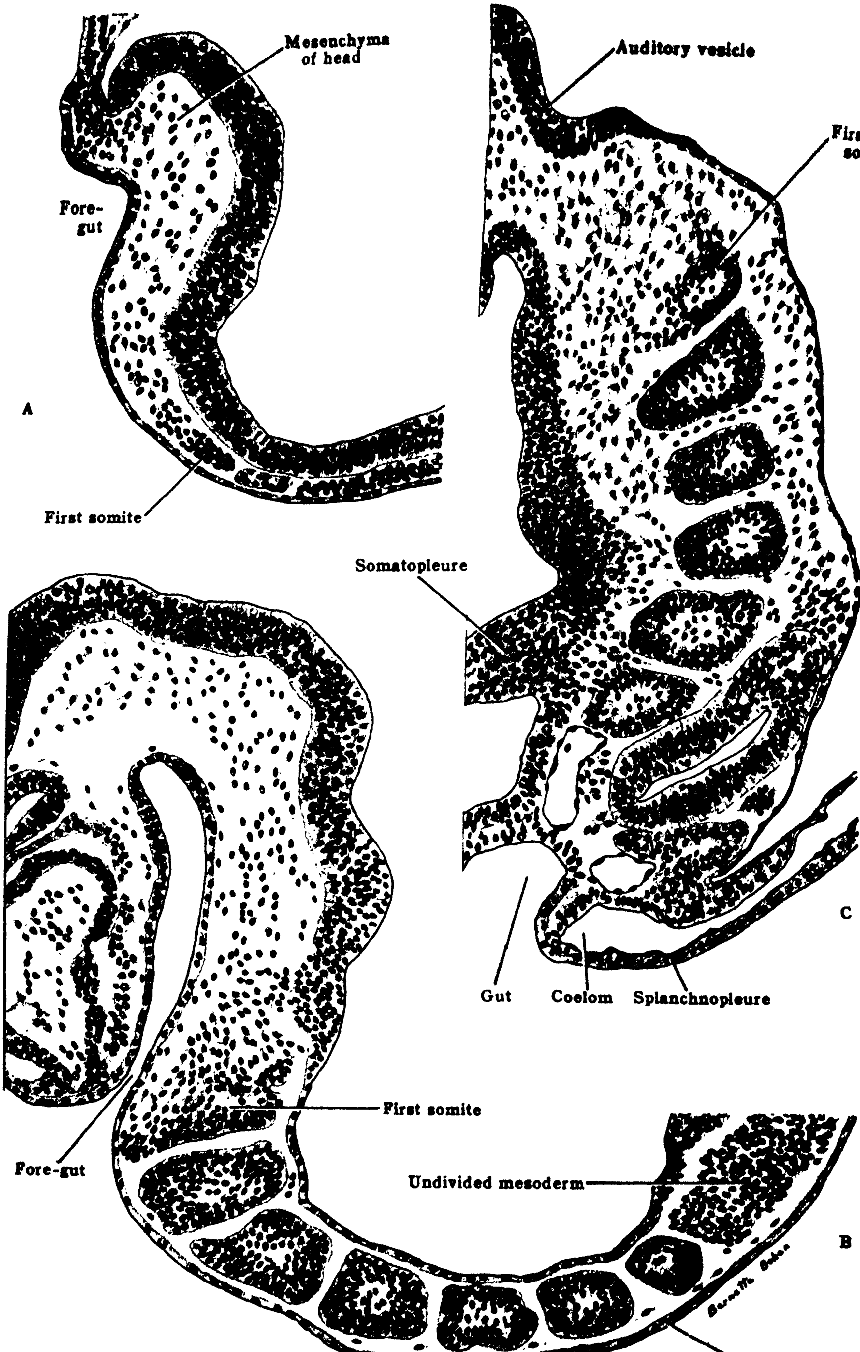


FIG. 18.—For descriptive legend see opposite page.

anterior end of the primitive streak. Each member of the pair appears as a localized denser area which grades off anteriorly into loose mesoderm, and which, posteriorly, is separated by a cleft from the undifferentiated caudal mesoderm. The second pair forms posterior to the first, and is likewise separated by a cleft from the undifferentiated caudal mesoderm. Additional pairs of somites form at more or less regular intervals, each new pair differentiating just posterior to the pair last formed, until a total of 65 pairs* has appeared (9). Continued and rapid proliferation of the mesoderm on each side of the primitive streak maintains a supply of undifferentiated cells. These push forward to about the level of the base of the notochord where the new somites are segmented off in regular succession. As a consequence of this manner of formation, the anterior somites are the oldest and, at any given stage, the most highly differentiated while the posterior somites are the youngest and the least differentiated.

Figure 18 shows in interesting fashion the way in which the "zipper action" by which the fore-gut is formed moves the location of the anterior intestinal portal steadily caudad. At the two somite stage the opening of the shallow fore-gut lies well ahead of the first somite. At the seven somite stage the anterior portal has moved backwards until it is just about at the level of the first somite. At the eleven somite stage it has reached approximately the level of the sixth somite. (The portion of the gut shown in Fig. 18C is mid-gut just caudad to the anterior intestinal portal.)

Because of the regularity with which successive somites develop, the number of somites in an embryo is a convenient means of stating its stage of development.

The primitive streak as a growth center.—The primitive streak is remarkable as being a region in which the three germ layers meet (Fig. 15). It is continuous dorsally with the ectoderm, laterally and posteriorly with

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* This figure is for the rat; the characteristic number may be slightly different for the mouse. There is some individual variation. Butcher states in his excellent paper on the somites of the rat that the first pair dedifferentiates and disappears at about the seven somite stage. We have found no evidence of such a dedifferentiation in our material. As may be seen from Figs. 18A, B, and C, the first somite can be traced clearly at least to the eleven somite stage.

FIG. 18.—Sagittal sections through somites. A. Embryo of 7 days 18 hours, with 2 somites formed. B. Embryo of 8 days 1 hour, 7 somites. C. Embryo of 8 days 11 hours, 11 somites. Because the embryo begins turning at about the 7 somite stage, the plane at which this 11 somite embryo is cut, although sagittal to the first 6 somites, is transverse to the mid-trunk region. Projection drawings (×150).

the mesoderm, and anteriorly with the head process (which is entodermal in nature and indistinguishably fused with the original proliferation of entoderm). Of these three, it gave rise to two, the mesoderm and the head process. It may be added that the somewhat distinct proliferation of mesoderm cells that produced the allantois occurred at its posterior end. Its own cells are undifferentiated in nature and cannot be classified as either ectoderm, entoderm or mesoderm. The only structure in primitive chordates possessing these characteristics is the dorsal lip of the blastopore, and it is probable that the primitive streak and the dorsal lip of the blastopore are homologous. Besides being a point of origin for new tissues, it is the center of a region of rapid growth. In sections it may be observed that the adjacent mesoderm is full of dividing cells, and as we have seen, cells from this region are continually pushed forward to give rise to somites anterior to the primitive streak, so that much of the increase in length of the embryo is due to growth in this region. We have already mentioned the head fold as a growth center. There are thus two regions of particularly active growth in the developing embryo, the primitive streak and the head fold (24). It is interesting to note one point of contrast between these two; namely, that the tissues in the head region are well advanced in differentiation while the tissues of the primitive streak region remain relatively undifferentiated.

The coelom.—Coincident with the formation of the somites in the paraxial mesoderm, the coelom or body cavity develops in the lateral mesoderm. It is formed by a division of this mesoderm into two layers, a dorsal or somatic layer adjacent to the ectoderm, and a ventral or splanchnic layer adjacent to the entoderm. The coelom is the space between the two (Fig. 17). Because the somatic mesoderm and the ectoderm are closely associated and undergo many foldings in common, it is convenient to designate the two layers together by the term somatopleure. For the same reasons splanchnic mesoderm and entoderm together are designated as splanchnopleure. It should be noted that the mesoderm and ectoderm of the somatopleure dorsal to the coelom are continuous with the similar layers in the amnion. The amnion, therefore, may also be classed as somatopleure. In like manner the mesoderm and entoderm of the splanchnopleure ventral to the coelom are continuous with the similar layers in a tissue which bounds the extra-embryonic coelom laterally. This tissue, therefore, may also be classed as splanchnopleure.

It has been previously stated that there is a mass of mesoderm in the mid-sagittal plane anterior to the fore-gut. This extends to right and left, across the front of the fore-gut and is continuous laterally with the lateral

sheets of mesoderm. It thus forms the base of a U of which the lateral mesoderm forms the sides. By about the four somite stage or slightly later, the coelom extends not only throughout the lateral mesoderm but also as a passage through this anterior mesoderm (Figs. 16 and 29). The coelom, also, is thus U-shaped. The whole posterior portion of the coelom opens laterally into the extra-embryonic coelom (Figs. 17 and 28). The anterior part of the coelom, on the other hand, forming the base of the U and extending as far posteriorly as the second somite, is separated from the extra-embryonic coelom by a partition of mesoderm. Much of this anterior portion of the coelom becomes the pericardial coelom, enclosing the heart. The connection between the anterior and the lateral parts of the coelom is called the pericardial-peritoneal canal (Fig. 28).

The relations of coelom and extra-embryonic coelom can be studied from the series of sections of a seven somite embryo shown in Figs. 23, A to G. The reader should note, however, that owing to the rapid development of the heart between the four and the seven somite stage, the pericardial coelom is already at this latter stage a more complicated cavity than when it first appeared.

Reichert's membrane.—The mouse embryo is protected during its development by three extra-embryonic membranes; namely, Reichert's membrane, the amnion and the yolk-sac. There is no essential difference between the amnion of rodents and that of other mammals. Reichert's membrane, on the other hand, is found only in the Rodentia, while the yolk-sac in this order has come to have rather unusual relations to other structures. The chorion, an important fetal membrane in most mammals is present in the mouse but remains small and, as a protective structure, unimportant.

To follow the development of Reichert's membrane we must go back to the 5½ day stage (Fig. 8). Except in the region of the ectoplacental cone, the embryo is bounded by the trophectoderm. This is continuous with the margins of the cone and is separated from the egg cylinder by the yolk cavity. Laterally its cells are in close contact with the maternal decidua, a contact so intimate in fact that in some cases it is impossible to tell whether a given cell is of embryonic or maternal origin. Ventrally it stretches across the remains of the uterine lumen, now filled with a degenerating mass of uterine epithelium. On the inner surface of the trophectoderm are a few widely separated entoderm cells.

A day later (Fig. 10) these entoderm cells have increased in number and form a uniform though not quite continuous layer over the inner surface of

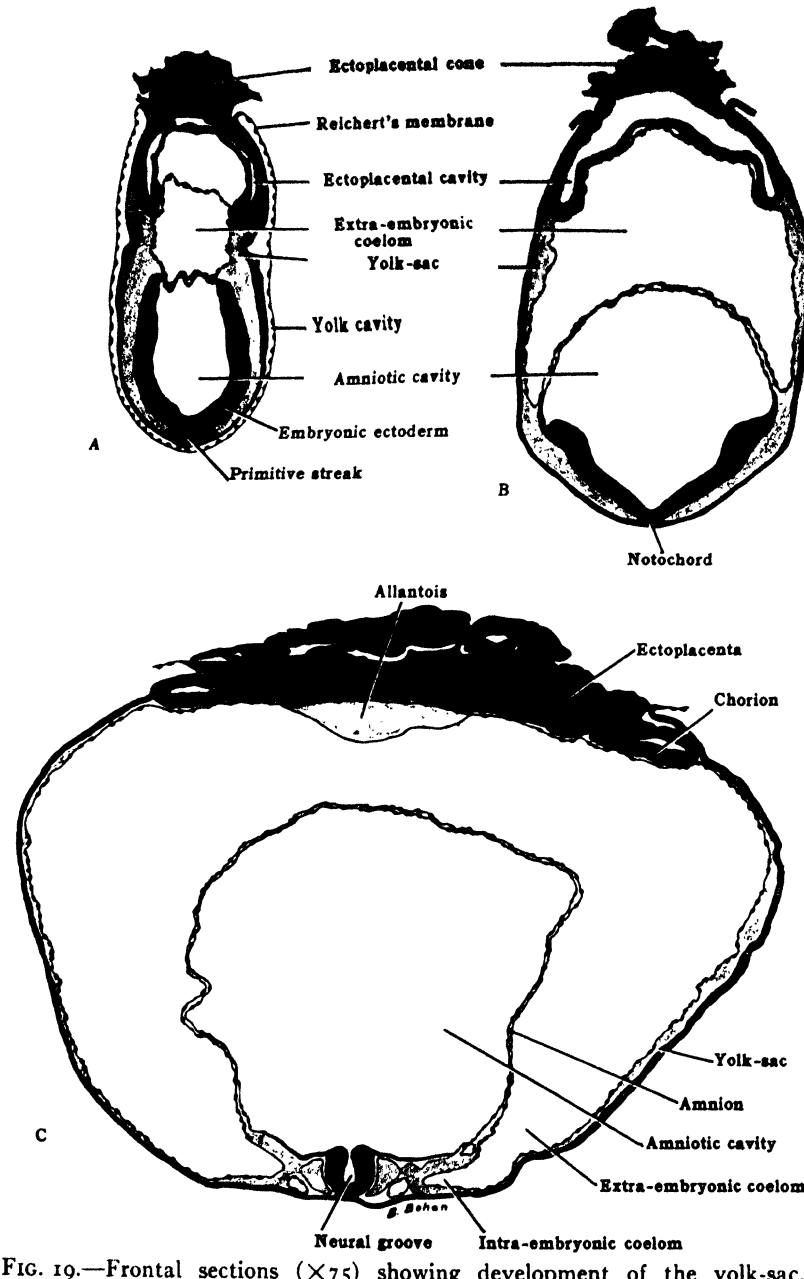


FIG. 19.—Frontal sections (×75) showing development of the yolk-sac. A. 7 days 6 hours. B. 7 days 10 hours. C. 8 days 10 hours, 9 somites, through 8th somite. Reichert's membrane omitted except in A.

the trophectoderm. Between the two cell layers there soon begins to appear a thin, non-cellular, pink-staining membrane called, after the man who first

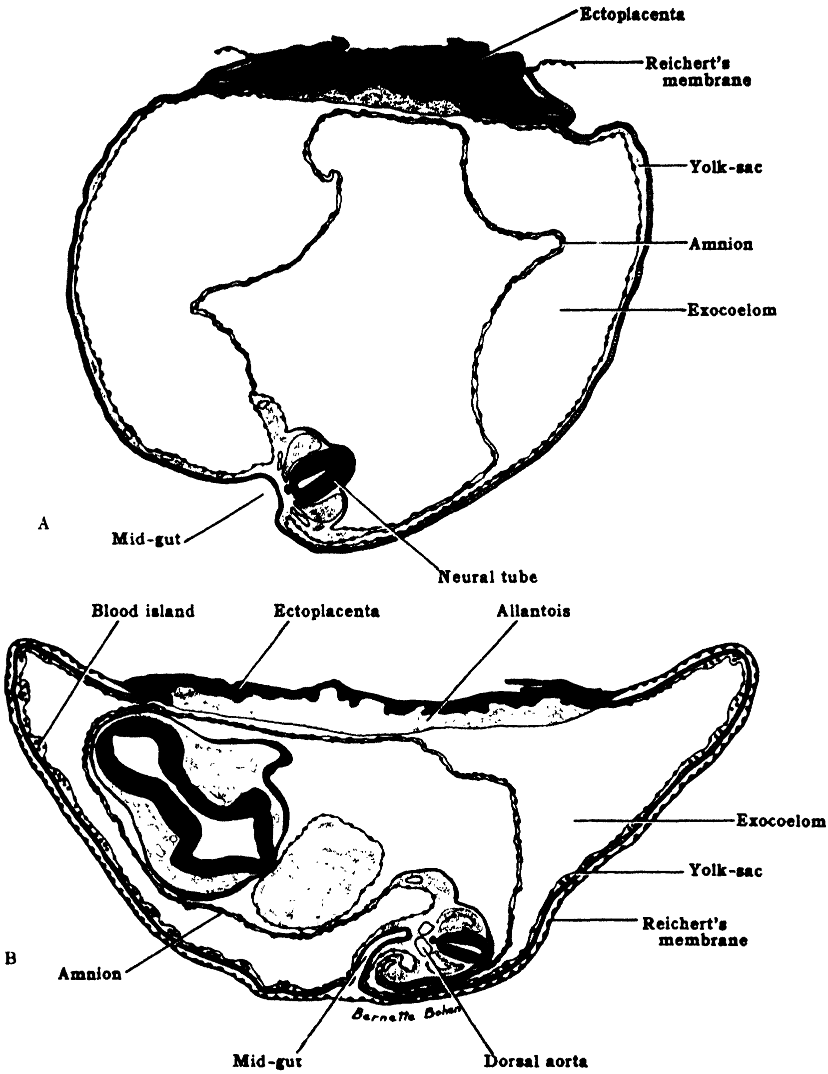


FIG. 20.—Frontal sections showing development of extra-embryonic membranes and formation of mid-gut. The sections are viewed from the head towards the tail, so that the right side of the embryo is on the left side of the drawing, and vice versa. A. 8 days 11 hours, 11 somites, through 9th somite. Reichert's membrane omitted. (×60.) B. 8 days 18 hours, 16 somites, through 9th somite. (×45.)

described it, Reichert's membrane. The first signs of it are often visible at the ventral extremity of the egg where there is apt to be a cluster of entoderm

cells. Perhaps this indicates that the entoderm cells produce it. In any case it soon becomes continuous over the entire inner surface of the trophectoderm. The fully developed membrane is of uniform thickness and, as can be demonstrated by dissection, surprisingly tough for so delicate a structure. Though non-living, it possesses the surprising property of being able to increase its area to keep pace with the growth of the embryo. Presumably this capacity for growth is dependent on the entoderm cells which are distributed at quite regular intervals over its entire inner surface.

The amnion.—The early stages of the development of the amnion have been described. Owing to the inversion of the germ layers, the amniotic folds have only a short distance to grow, and amnion formation is consequently precocious in the mouse as compared with most other mammals (Figs. 9B and E). For the same reason, the area of the amnion at first is small. It expands rapidly, however, to accommodate the growing embryo and by 8 days it forms a large sac over the embryo's entire dorsal surface (Figs. 22 and 25C). In the later stages of development the embryo floats free in the amniotic cavity attached only by the umbilical cord.

The yolk-sac.—The mammalian ovum contains virtually no yolk. The mammals are, however, descended from reptilian ancestors in whose eggs yolk was abundant, and this long period in their evolutionary history has left an indelible impress on mammalian development. Most striking, perhaps, is the development of a yolk-sac so similar in many details to the reptilian yolk-sac as to be unmistakably homologous. As is often the case with vestigial structures, this has been modified in different ways in the different groups of animals that have inherited it. In the rodents it gives rise to a membrane enveloping the embryo and possessing the dual function of protection and, during the middle stages of development, of absorbing nourishment from the mother.

The yolk cavity of the mouse may be defined as the cavity derived from the original segmentation cavity or blastocoele and lying between the egg cylinder and Reichert's membrane (Fig. 19A). The yolk-sac is only a part of the boundary of this cavity; namely, that middle portion of the egg cylinder wall which is composed of mesoderm and entoderm, or in other words, of extra-embryonic splanchnopleure.*

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* It should be noted that in many mammals, e.g., the pig, the allantois as well as the yolk sac are derived from splanchnopleure. This is not the case in the mouse. In this species the yolk-sac, as we are using the term, and the extra-embryonic splanchnopleure are identical.

At 7¼ days the extra-embryonic splanchnopleure or yolk-sac is a structure of limited area forming the central or ectoderm free portion of the egg cylinder wall (Fig. 19A). While small at first, it is an area of rapid growth and by 8 or 8½ days forms an extensive membrane enveloping the amnion and a greatly enlarged exocoelomic cavity (Figs. 19B and C). The whole embryo changes its shape in the process, the egg cylinder becoming an ovoid and the ovoid a sphere. At 8 days the yolk-sac is still attached to the embryo along a band that runs anterior to the opening of the fore-gut and posterior to the opening of the hind-gut, so that most of the ventral surface of the embryo is outside it (Fig. 22). After the mid-gut has formed, however, this portion of the embryo, too, is enveloped by the yolk-sac (Fig. 20). The details of this process will be described later.

The blood islands.—Associated with the yolk-sac splanchnopleure in all species in which it occurs are structures known as the blood islands. These appear in the mouse at 7½ days as thickenings in the inner or mesodermal layer of the yolk-sac about which they form an irregular girdle (Fig. 16). As the name implies, the blood islands give rise to part of the circulatory system. The peripheral cells differentiate to form the endothelium of a system of blood vessels encircling the yolk-sac while the inner cells become primitive blood corpuscles that circulate in the embryonic blood stream.

Changes in the uterus.—Implantation is accompanied by a rapid growth of the uterine mucosa adjacent to the implantation site to produce a definite swelling, the decidual swelling. For a while the uterine crypt containing the embryo maintains its connection with the uterine cavity, but by about 7½ days the growth of the decidua has blocked this off so that the cavity containing the embryo is separated from the main lumen (Fig. 4B). The bridge of tissue thus formed dorsal to the ectoplacental cone will later become part of the placenta. Further growth of the decidua constricts and finally, by about 8 days, completely closes the uterine lumen dorsal to the embryo except for one or more small isolated chambers (Fig. 21). On each side of the decidual swelling the uterine lumen remains open, but at this period in development there is no continuous passage throughout the length of the uterus. A little later a continuous lumen is reestablished, but the new lumen is on the opposite side of the decidual swelling from the old, passing ventral instead of dorsal to the embryo. An early stage in this reestablishment of the lumen may be seen at about 8 days (Fig. 21). The epithelium lining the lumen on each side of the decidual swelling has grown in between the muscle layers and the decidua ventral to the embryo. The extreme limits of this growth consist of a double but unsplit layer of epithelium. In

the slightly older epithelium nearer the lumen the two layers have split so that two wedgeshaped spaces extend from the lumen between decidua and muscles on each side of the decidual swelling. In course of time the wedges penetrating from the two sides meet ventral to the embryo, thus completing the formation of the new lumen.

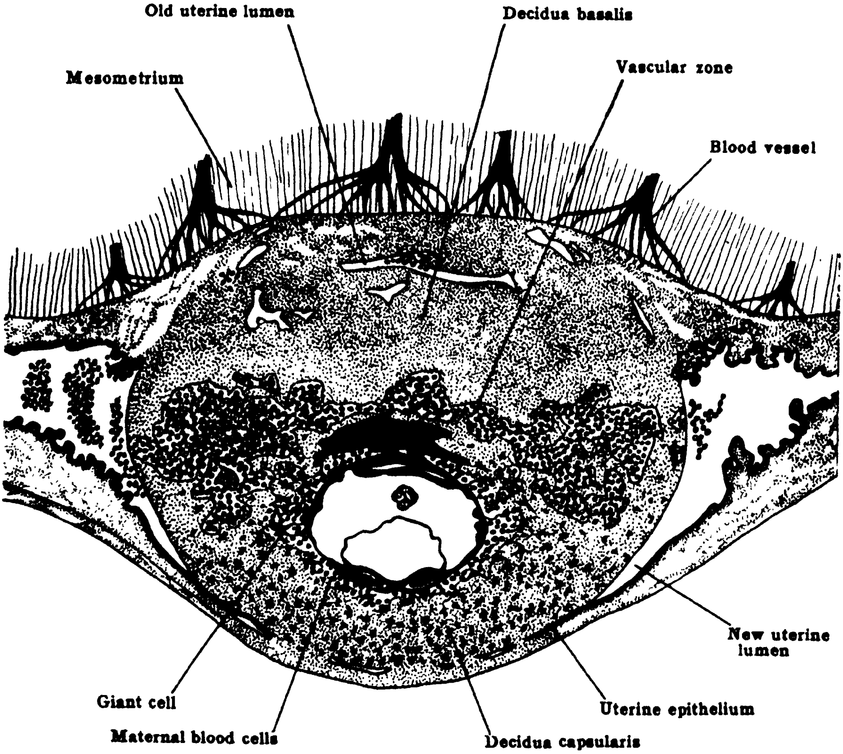


FIG. 21.—Longitudinal section (partly diagrammatic) of uterus at site of implantation of 8 day 6 hour, 5 somite embryo. Cut parallel to mesometrium.

Besides the changes in the uterine lumen there are interesting changes in the histology of the decidua. Starting as a relatively homogeneous tissue, different zones differentiate within it, each with its characteristic structure. As many as six zones have been distinguished (33), but for our purposes it will suffice to note no more than three (Fig. 21). Ventrally there is an antimesometrial zone or decidua capsularis characterized by large bi-, tri- or tetra-nucleate cells.* The individual nuclei in this zone as well as the cells

* In the rat this region is characterized by bi-nucleate cells. Krehbiel (33) states that more than two nuclei do not occur.

are larger than elsewhere in the decidua, and this together with the grouping of the nuclei gives the zone a very characteristic appearance. It will be noted that it lies between the embryo and the new uterine lumen. With the growth of the embryo it becomes stretched until, in the later part of the gestation period, it is hardly more than a thin membrane separating embryo and lumen. Dorsally there is a mesometrial zone, or decidua basalis, whose cells at 8 days still closely resemble those of the unaltered mucosa. It later contributes to the formation of the placenta. Between the antimesometrial and mesometrial zones is an intermediate or vascular zone characterized by the presence of numerous irregular endothelial-lined blood spaces or sinusoids. Its cells tend to be multi-nucleate like those of the decidua capsularis.

The nourishment of the embryo.—The source from which the embryo derives its nourishment during its earliest growth period is somewhat uncertain, but it is not unlikely that the degenerating cells of the uterine epithelium that originally lined the implantation chamber serve as a source of food. The epithelium is sloughed off and begins to undergo degenerative changes at just about the same time that the first real increase in size of the embryo is to be noted. At the mesometrial pole of the embryo at 4½ days may be seen droplets of secretion that contain perhaps an enzyme concerned with the digestion of the epithelial cells (Fig. 6). This stage in the nourishment of the embryo is brief; by 5½ days only a remnant of the epithelial cells remains (Fig. 8).

At the same time a new source of nourishment makes its appearance. It has already been stated that the intermediate zone of the decidua contains numerous blood-filled sinusoids. At 5½ days these begin to rupture, pouring their contents into the lumen surrounding the embryo. In a very short time the embryo is completely bathed in maternal blood. It has recently been shown that this blood is not stagnant as was once supposed, but that it remains a part of the maternal circulation. In certain experiments with the rat it was found that there is a complete replacement every twenty minutes (13).

The maternal blood is separated from the embryo proper by Reichert's membrane, the yolk cavity and, in later stages, by the yolk-sac. Reichert's membrane probably plays an entirely passive rôle in the transportation of nutrient substances from the maternal blood to the embryo, acting simply as a semi-permeable membrane. The yolk-sac, on the other hand, probably actively absorbs the food material. This is particularly true after the blood islands which girdle the yolk-sac have developed into a capillary network and

after the embryonic circulation is established. By the time this occurs the yolk-sac has become pressed against and partly fused with Reichert's membrane, obliterating the yolk cavity (Fig. 20B). The embryonic yolk-sac circulation is thus brought very close to the maternal circulation, and the yolk-sac is established as "an organ of exchange whose importance is not secondary to that of the allantoic placenta" (13). It is interesting to observe that in the rodents the yolk-sac has thus recovered in full measure the rôle as an organ of absorption which it possessed in the reptiles, with, however, the important difference that the material absorbed comes from the maternal blood instead of from yolk deposited within the egg.

The sinusoids in the intermediate zone of the decidua extend from the decidual cavity containing the embryo clear to the periphery of the decidual swelling where this borders on the uterine lumen. Beginning at about 7½ or 8 days there is bleeding into the uterus from these peripheral sinusoids (64, 66). At about 10 days some of this blood finds its way into the vagina, persisting there for 3 or 4 days (57). It is a convenient early sign of pregnancy.

In the later stages of development the decidua basalis, the ectoplacental cone, the chorion, and parts of the allantois fuse to give rise to a true placenta which thereupon assumes a major rôle in transferring nutritive material to the embryo.

The giant cells.—A conspicuous feature in sections of mouse embryos of 6 to 14 days is the presence of certain remarkably large cells lying between Reichert's membrane and the decidua. These are the so-called giant cells (Fig. 21). Because of the early and close fusion of embryonic and maternal tissues in the region which the giant cells later occupy, their origin is difficult to determine and has been the subject of extended debate (3, 22, 48 and others). Some authors believe that they are derived from the decidua, others that they come from the trophectoderm. Their function likewise has been the subject of much discussion. Our own observations, briefly presented below, seem to us to be fairly conclusive on a few points, but to leave others still in doubt.

It is convenient to distinguish three types of giant cells. The first large and unmistakable giant cells to appear are at the ventral extremity of the embryo (Figs. 6 and 8). They are quite evidently derived from the trophectoderm. Already quite large at 5½ days, they become, relatively speaking, enormous by 7 days at which time they have penetrated for some distance into the remains of the implantation cavity ventral to the embryo. These are primary giant cells. The trophectoderm cells lateral to the egg cylinder

probably also give rise to similar though somewhat smaller primary giant cells.

A second and much more numerous group of giant cells is quite probably derived from the ectoplacental cone. Already at 5 days cells may be seen growing down outside the trophectoderm from the region of the future cone (Fig. 7). Later, when the embryo is surrounded by maternal blood, these become long strands of cells extending down, within the blood or along the inner surface of the decidua, from the cone towards the ventral extremity of the egg cylinder. At first small, these cells increase in size and at 8 days form a loose meshwork of large cells whose long protoplasmic processes extend across the blood filled space between Reichert's membrane and the decidua (Fig. 21). Other similar cells may be seen adjacent to the ectoplacental cone. These are the secondary giant cells. At 8 days their continuity with the cells of the ectoplacental cone is still quite obvious. While this is the probable origin of the majority of the giant cells, the possibility that at least some of them are derived from the decidua is not ruled out. It should be pointed out that the division between primary and secondary giant cells is partly arbitrary; the trophectoderm and the ectoplacental cone are continuous structures, and cells from near the line of junction might be said to give rise to either type. One obvious function of the giant cells is to anchor Reichert's membrane to the decidua. They quite probably have other functions also, but what they are is uncertain.

The third class of giant cells consists of the so-called symplasia. These cells, individually conspicuous but never very numerous, are multinucleate cells first appearing in the decidua adjacent to the embryo at 7 or 7½ days. The number of nuclei per cell is extraordinary, mounting into the dozens by 8 days. The nuclei are dark staining and closely packed. The origin and function of the symplasia is uncertain.

The seven somite embryo.—In embryos from genetically vigorous stock, the seven somite stage is reached at about 8 days. As thereafter the embryo begins a series of important changes, it will be useful to review here the development attained at this point (Figs. 22 and 23). In sagittal section the embryo is seen to form a letter S (facing to the left in Fig. 22) with the head region convex, the trunk region concave towards the dorsal surface. In transverse section, whereas the embryo was formerly conspicuously cup-shaped with the ectoderm on the inside, it has now flattened out, in fact in the regions of the fore- and hind-gut the entoderm has become the inner layer. The neural groove, deep and well developed, is still open dorsally though in the mid-trunk region the walls are quite close together. Cephalad,

precocious growth of certain parts of the neural groove ectoderm indicates early differentiation of the brain. The hind-gut is small, but there is a deep fore-gut, and the heart, just anterior to the fore-gut, is a conspicuous structure. No mid-gut has formed. The allantois has almost reached the chorion, in fact in some embryos at this stage has already reached and fused

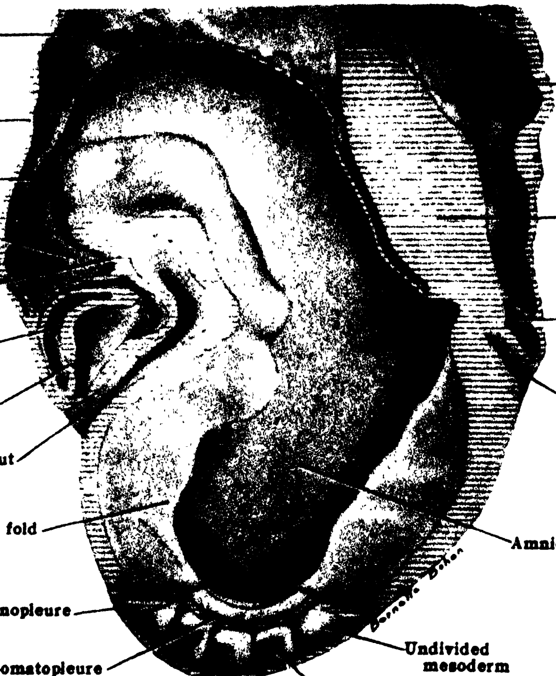


FIG. 22.—Drawing of reconstruction of 8 day 1 hour, 7 somite embryo. The reconstruction is cut in the mid-sagittal plane and only the right half shown except at the ventral extremity where the last 4 somites and part of the undivided mesoderm of the left side are included. Cut areas are shown by horizontal shading. (×75.)

with it. The amnion, and the yolk-sac plus the chorion, form a double, arched roof over the whole dorsal surface of the embryo. The blood islands appear as a conspicuous hummocky band around the inner surface of the yolk-sac. Within the embryo blood vessels have begun to form.

The tail fold.—The hind-gut, though much later to appear than the foregut, soon overtakes it in development. In ten somite embryos the two are of approximately equal size (Fig. 31). A necessary concomitant of hind-gut growth is the appearance of a tail fold; the gut entoderm pushes the overlying ectoderm and mesoderm ahead of it away from the yolk-sac wall.

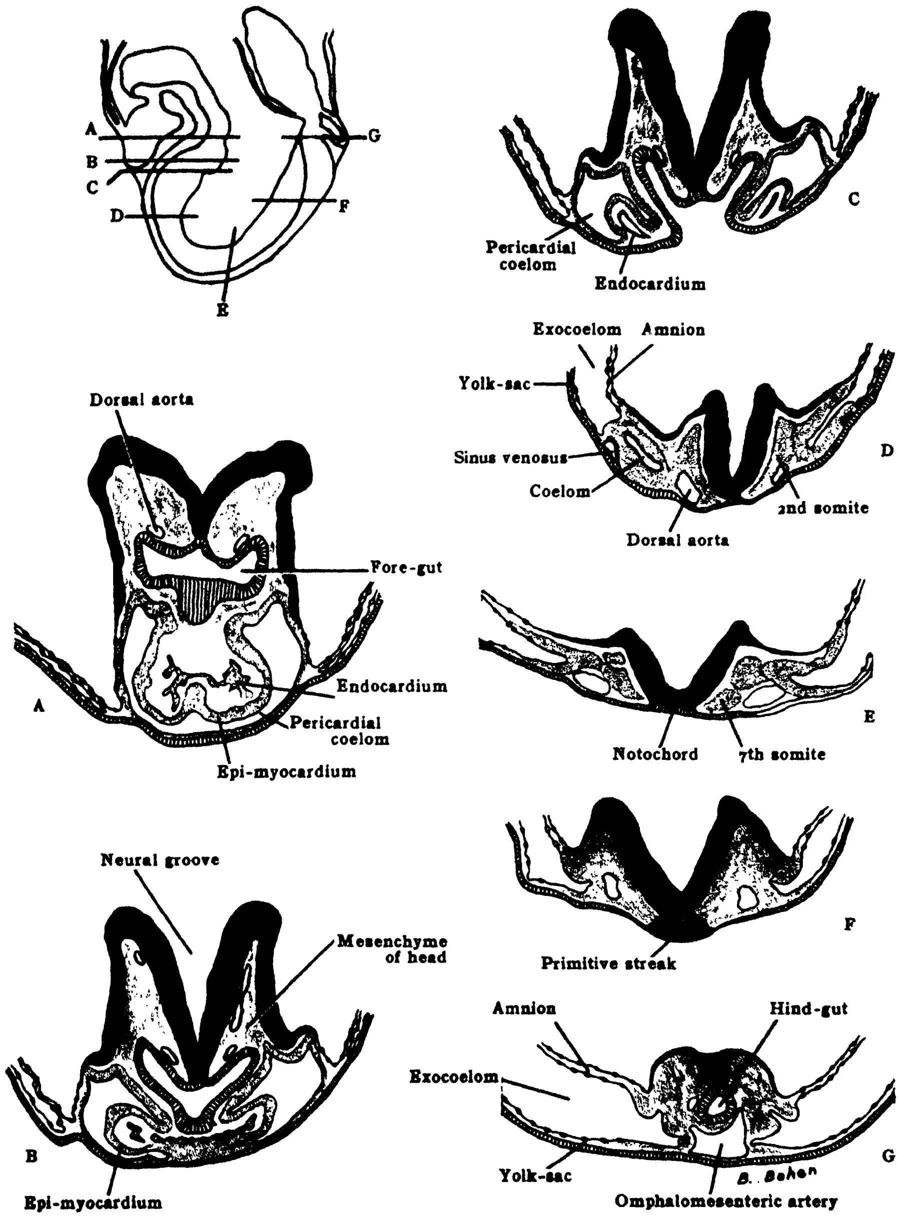


FIG. 23.—Sections transverse to neural groove of 8 day 1 hour, 7 somite embryo. All except E are from the embryo shown in figure 22. The location of each section is indicated on the key diagram. (×90.)

A beginning of this process can be seen in six somite embryos (Fig. 16), and in ten somite embryos the tail fold is well developed (Figs. 26A and 27). The process is strictly comparable to the formation of the head fold except for one interesting difference; whereas the head fold lies entirely within the amniotic cavity, the tail fold lies only partly within it. The ventral surface of the tail fold is in the exocoelom. This is because in its growth away from the yolk-sac it pushes the base of the allantois and the adjacent margin of the amnion ahead of it. The amnion remains attached to its caudal and lateral walls, and only its dorsal surface is within the amniotic cavity (Fig. 24).

The turning of the embryo.—Almost immediately after the seven somite stage the embryo begins a process of turning which results in a reversal of the curvature of the whole trunk region. Thus instead of being S-shaped the embryo becomes C-shaped with the ventral surface everywhere on the inside of the C. The turning begins in the head and tail folds, and consists of a rotation of each along its long axis, or in other words, on axes parallel to the fore- and hind-guts (Figs. 24–28). Viewing each fold from its cephalic toward its caudal end, the direction of rotation is clockwise in each case. Of course, both folds cannot be viewed in this direction from any one point, because of the curvature of the embryo. Viewed from the mesometrial pole, in sections the turning of the head fold appears to be clockwise, of the tail fold counter-clockwise (Fig. 24).

At first the turning is confined to the head and tail folds; the mid-trunk region, still firmly attached to the yolk-sac, remains in its original position. At about 8½ days, and at about the eleven or twelve somite stage, the mid-trunk region turns also. The process is sudden. Transverse sections of the trunk region at about this period show it to be either turned or not turned (Figs. 19C and 20A). It is quite possible that after the growth of the head and tail folds reduces sufficiently the attachment of the trunk region to the yolk-sac, this region snaps over like a spring whose tension has come to exceed the forces holding it. Some time elapses after the turning of the mid-trunk before the head and tail regions complete their rotation, which eventually amounts to a full 180°. Essentially, however, by about 9 days the embryo has become concave towards the ventral surface (Figs. 26B and C).

The mid-gut.—The turning of the mid-trunk region automatically results in the formation of the mid-gut. Prior to turning, the two sheets of embryonic splanchnopleure in the mid-trunk region extend straight out from the sides of the embryo, forming a virtually plain surface (Fig. 19C). There is thus no indication of a mid-gut. When the mid-trunk region turns suddenly towards its left side, the two sheets are pulled after it, forming between them

a groove which is continuous anteriorly and posteriorly with the fore- and hind-guts. This groove is the mid-gut (Fig. 20A). The two sheets of splanchnopleure rapidly draw closer together (Fig. 20B), and at the nineteen somite stage, which may be reached as early as 8¾ days, have fused distally to form a closed tube.

The heart.—It will be remembered that in 7½ day embryos there is a small region of mesoderm anterior to the fore-gut (Fig. 15). This forms the base of a U of which the two lateral sheets of mesoderm form the sides. Within this U the coelom develops and is, therefore, itself U-shaped. The base of the U, and the two sides approximately as far caudad as the second

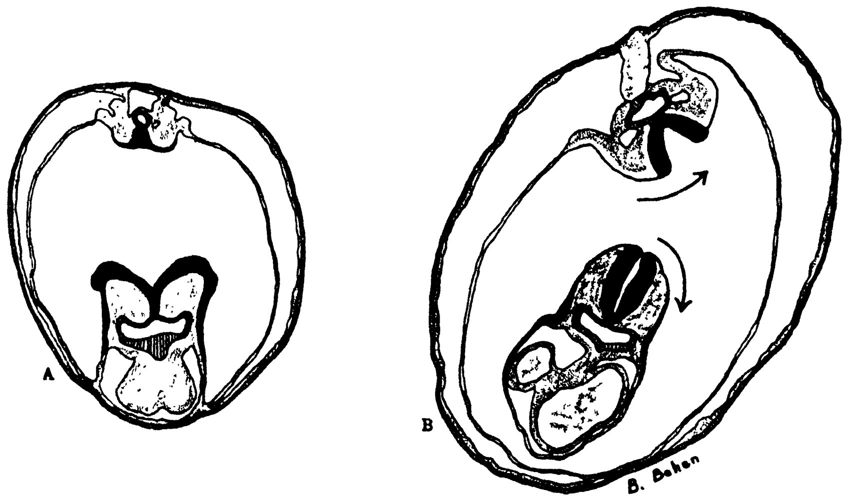


FIG. 24.—Transverse sections showing the turning of the embryo. A. 8 days 1 hour, 7 somites. B. 8 days 10 hours, 10 somites. (×50.)

pair of somites, contain that portion of the coelom which ultimately encloses the heart and which, therefore, is known as the pericardial coelom (Fig. 22). The curved shape of the pericardial coelom in cross section in Fig. 22 should not be confused with the U-shape of the pericardial coelom as a whole.

The heart is derived from the splanchnic mesoderm which forms the ventral wall of the pericardial coelom (Fig. 29). In five somite embryos this mesoderm has differentiated into two layers. Adjacent to the pericardial coelom is a thick, continuous layer, known as the epi-myocardium because it will give rise both to the heavy muscular layer of the heart wall (myocardium) and to its outer covering (epicardium). Between the epi-myocardium and the underlying entoderm are a number of irregular cavities which later fuse to form the cavity of the heart. The lining of these cavities is the endocardium.

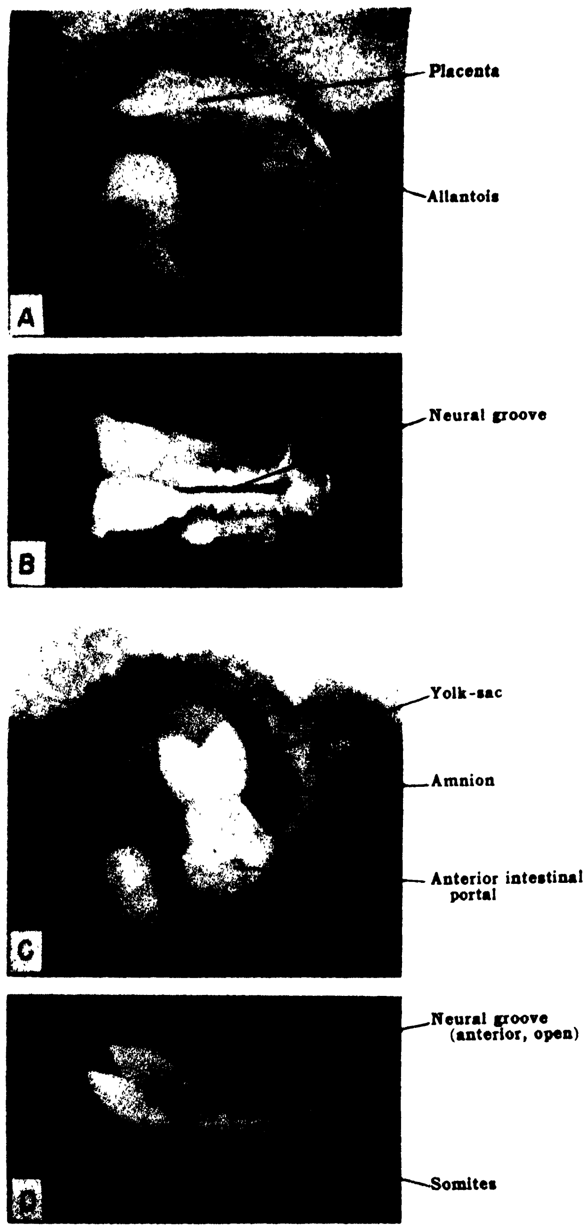


Fig. 25.—Photographs (×25) of mouse embryos. A. Lateral view of 7 day 18 hour, 6 somite embryo, with decidua and most of yolk-sac dissected. B. Dorsal view of same embryo, amnion also dissected. C. 10 somite embryo, age 9–9½ days. Slightly retouched. Embryo from inbred stock. D. Same embryo as C, dorsal view, amnion removed.

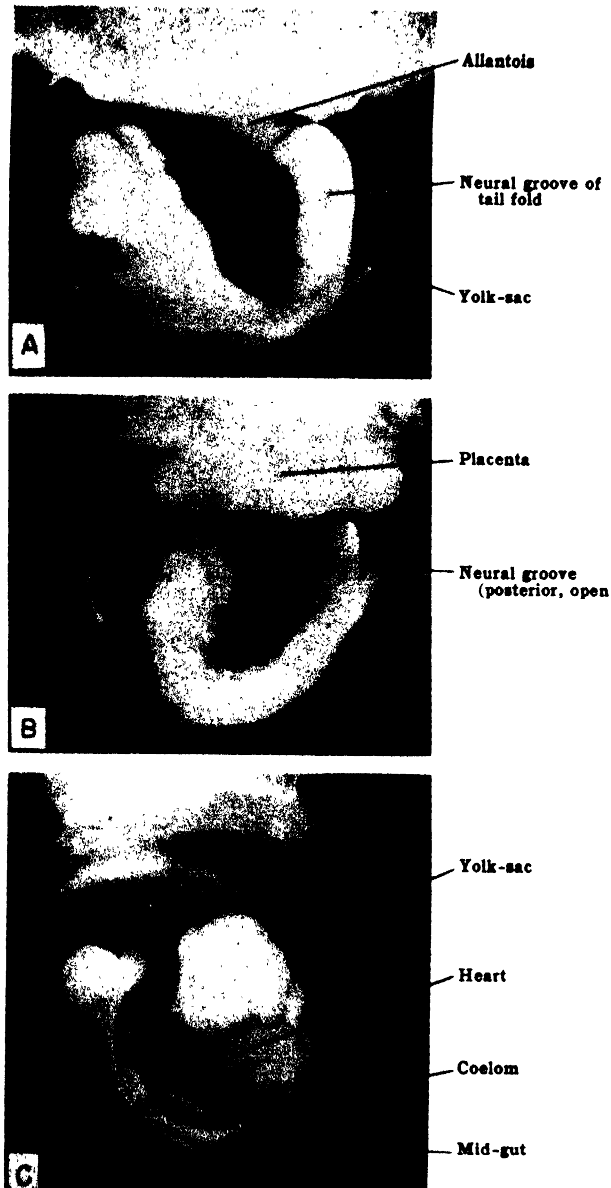


FIG. 26.—Photographs (×25) of mouse embryos. A. 13 somite embryo, age 9¼–9¾ days, from inbred stock. B and C. 14 somite embryo, age 8 days 22 hours. Note the greater degree of turning of this embryo, particularly in the mid-trunk region, as compared with the one in A.

Because of its relation to the U-shaped pericardial coelom, the heart is itself a U-shaped structure at this stage* with the base of the U lying just cephalad to the anterior intestinal portal (Fig. 30). As the intestinal portal

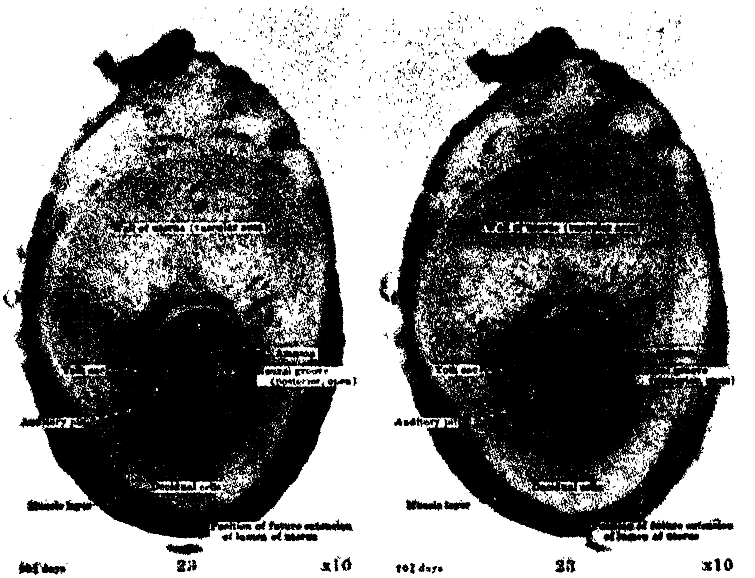


FIG. 27.—Stereoscopic photograph (×10) of rat embryo, age 10¾ days. (*From Long and Burlingame.*)

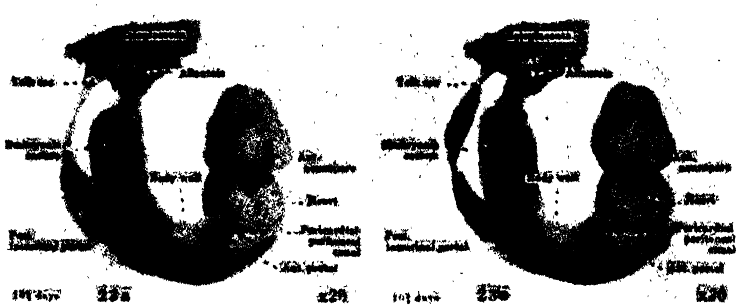


FIG. 28.—Stereoscopic photograph (×20) of rat embryo, age 10¾ days. Same embryo as Fig. 27, more completely dissected. (*From Long and Burlingame.*)

moves caudad due to the "zipper action" which causes the progressive folding together and fusion in the mid-ventral line of the entoderm which bounds

* In many vertebrates the heart originates as two entirely distinct primordia which later fuse. As has been clearly shown by Goss (16) and by Burlingame and Long (8), this is not the case in the rat. Our observations indicate that the condition in the mouse corresponds closely to that in the rat.

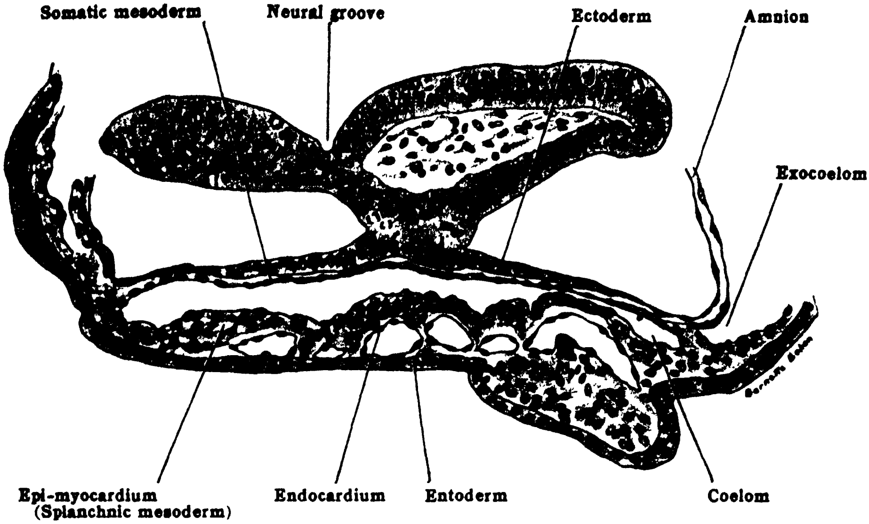


FIG. 29.—Slightly diagonal transverse section through median endocardial primordium (see Fig. 30), just cephalad to fore-gut. Embryo of 8 days 6 hours, 5 somites. Projection drawing (×150).

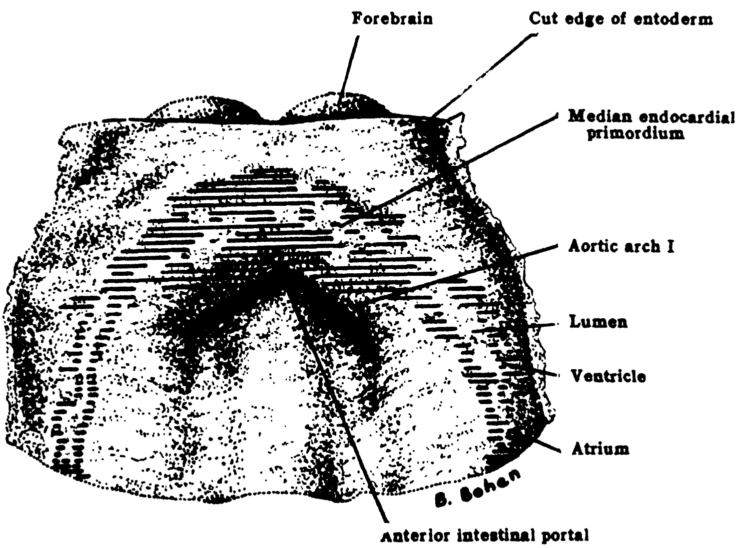


FIG. 30.—Diagram of the fore-gut region viewed from the ventral surface, showing distribution of the endocardium. Endocardial tissue is represented by horizontal lines. Rat embryo of 9 days 16 hours, 3 somites. (*Modified after Goss.*)

it, the sides of the U are likewise brought into approximation and fused together in the mid-ventral line. The endocardium is thus transformed from a U-shaped structure into a single tube. At the three somite stage (in the rat) the different regions of the heart are not clearly set apart, though a slight constriction serves to mark the boundary between the atrium and the ventricle. As a result of subsequent foldings of the endocardial tube the different regions of the heart are clearly differentiated (Fig. 31).

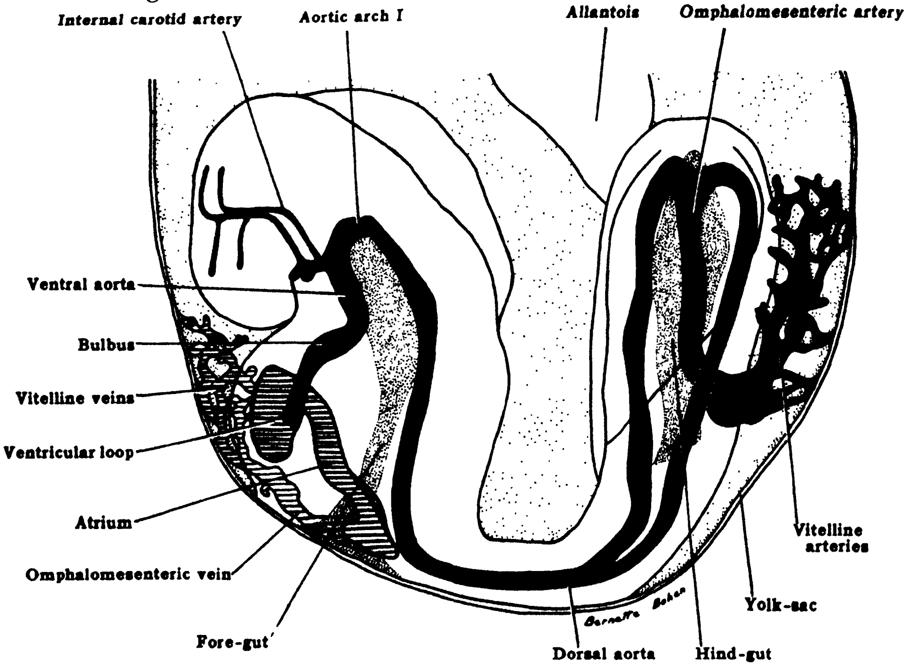


FIG. 31.—Diagram of the circulatory system in an 8 day 10 hour, 10 somite embryo. The head and tail folds of this embryo have begun to turn but there is as yet no turning in the mid-trunk region. Traces of the allantoic veins are present but are not shown as they do not yet form a continuous channel. (×64.)

Blood vessels.—In ten somite embryos, still in the process of turning, a number of blood vessels have become established (Fig. 31). The dorsal aorta at this stage is a paired vessel running the length of the trunk. It connects anteriorly with the heart by way of the aortic arches and the ventral aorta. Posteriorly its two halves fuse at the caudal extremity of the hind-gut to form the single, median, omphalomesenteric artery. This runs cephalad for a short distance ventral to the hind-gut and then turns away from the embryo towards the inner surface of the yolk-sac on which it spreads out into a network of capillaries. These capillaries are derived from

the blood islands. At this stage actual blood channels have not appeared in most of the blood islands, but when these are established, a capillary network is formed encircling the yolk-sac. Blood is collected from this network anteriorly by the paired, omphalomesenteric veins which convey it back to the heart. When the heart starts beating, this system of blood vessels provides a generous circulation through the yolk-sac which serves at this time as the principal organ for the procurement of food from the mother.

Change in shape of the yolk-sac.—When the embryo starts turning, the yolk-sac and ectoplacental cone form a slightly flattened sphere (Fig. 19C). When turning has been completed, these bounding structures of the embryo shortly assume the form of a slightly saucered-out hemisphere (Fig. 20B). The ectoplacental cone becomes flattened and then dorsally concave, and the yolk-sac adjacent to the cone pushes outward into the porous, blood-filled vascular zone of the decidua. The embryo meantime, still attached to the yolk-sac by the walls of the mid-gut, tips over so that it lies with its left side adjacent to the yolk-sac, its right side facing the placenta.

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Chapter 2

REPRODUCTION

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Since the processes of reproduction are very similar in both mouse and rat, the following discussion includes data from both species. Where no mention is made of the species, it may be assumed that the mouse is the animal referred to. Certain phases of reproduction in the mouse and rat are dealt with much more thoroughly than others. The references listed below contain important material not covered in this chapter.

Anatomy of the male and female reproductive systems: Chapter 3 of this book.

Endocrines and reproduction: Parkes (102), Allen (6), Young (137).

Viability and transport of spermatozoa: Chapter by Hartman, in Allen (6).

Spermatogenesis: Hays (67); see also references in Chapter 3 of this book.

The vaginal plug.—Copulation in the mouse and rat is accompanied by the formation of the vaginal plug, the presence of which is thus a convenient sign that mating has occurred. The plug is formed by a mixture of the secretions of the vesicular glands and the coagulating glands of the male (134, see also p. 137), and in the mouse usually fills the vagina from the cervical canal to the vulva, from which it may even protrude. Occasionally smaller, less conspicuous plugs are formed, a condition particularly common in the case of matings at the first post partum estrus. Plugs in the mouse usually persist for 18 to 24 hours, occasionally for as long as 48 hours, after which they are sufficiently loosened, probably as the result of leukocytic action, to fall out almost entire (100).

Gestation.—The gestation period in the non-suckling mouse is usually 19 or 20 days (36, 73, 97, 100). The frequency distribution of gestation periods of different lengths for two inbred strains of mice is given in Table 1.

Hybrid stocks tend to have shorter gestation periods than inbred stocks. The normal gestation period in the rat is slightly longer than in the mouse, ranging from 21 to 23 days (69).

Table 1
LENGTH OF GESTATION PERIOD IN THE C57 BLACK AND DBA STRAINS (FEKETE, UNPUBLISHED DATA)

Days	Number of Litters	
	C57 Black	dba
18	1	0
19	41	10
20	51	84
21	6	24
22	0	3

Birth of litters most commonly occurs at night (92). There is a decided maximum in the number of births between midnight and 4 A.M., but parturition between 4 P.M. and midnight is not uncommon. Altogether, of 164 timed births, two thirds occurred between 4 P.M. and 4 A.M.

An estrus occurs about 20 hours after parturition, and while fertile matings at this time are not common in some stocks of mice (35) unless the newly arrived litter is killed at birth, they occur quite regularly in other stocks. Thus lactation and gestation may proceed simultaneously. Under these circumstances the gestation period is lengthened, the extent of the lengthening being slightly correlated with the number of suckling young With only one or two young suckling, the prolongation does not exceed 7 days, with three or more young suckling prolongations up to 12 or 13 days are not uncommon. The maximum recorded is 16 days (20, 47, 51, 136). Kirkham (73) has shown that the prolongation is due to a delay in implantation, which, instead of occurring during the fifth day post coitus as normally, occurs on some later day, the embryos meantime lying free in the uterus. Mating may occur during pregnancy (33), but that such matings are accompanied by ovulation is open to doubt.

Litter size.—Litter size differs greatly with the strain, with the age and condition of the mother, and with order of the litter. Bittner (10) gives the data reproduced in Table 2 which shows the relation between order of litter and litter size for the highly inbred A strain.

Many hybrid animals produce litters considerably larger than those produced by the A strain. Grüneberg (61) reports taking 19 healthy embryos just short of term from one hybrid female. Gates (56) reports an average size of 7.4 with a range of 2 to 12 for 106 litters in a random bred strain. This is fairly typical for many strains.

Table 2
ORDER OF LITTER AND LITTER SIZE IN A STRAIN MICE (FROM BITTNER)

No. of Litter	Mean Litter Size
1st.	5.13 ± 0.08
2nd	6.35 ± 0.09
3rd	6.46 ± 0.09
4th	6.21 ± 0.10
5th	5.53 ± 0.11
6th	4.62 ± 0.13
7th	4.01 ± 0.14
8th	3.50 ± 0.34
Total	5.68 ± 0.04

The number of corpora lutea formed at the time of the last mating is, with possible rare exceptions, identical with the number of eggs ovulated. This number is quite highly correlated with parity (order of litter) and with weight of the mother, but only slightly correlated with age (88). It may be used as an index of pre-natal mortality. MacDowell (86) finds that 33.9 per cent of the ova that come to maturity are not represented by living young at birth. This is an average figure based on results from several strains; there are considerable strain differences. Thus the dba strain shows a higher prenatal mortality than the C57 black strain (Fekete, unpublished data).

There is evidence that mouse ova may split to produce uniovular twins, and that these may come to term, but the phenomenon is certainly rare (15, 27, 59, 109, 129).

Sex ratio.—According to genetic theory, males produce equal numbers of male-producing and female-producing sperm, so that, except for a possible difference in functional capacity of the two types of sperm, or a possible selective effect of prenatal mortality, the sex ratio at birth should be 1:1. MacDowell and Lord (89) have recorded the sex of 106 litters of mice in which the number born was no less than the number of corpora lutea, and hence in which prenatal mortality is ruled out. Their count showed

261 males and 262 females, an almost exact 1:1 ratio. MacDowell and Lord (90) also present evidence that there is no continuous selective elimination of one sex or the other before birth.

An alteration in the sex ratio through excessive breeding of the fathers and through treatment of the fathers with alcohol has been both claimed and denied (28, 57, 87, 104 and others). An effect through injection of the uterus with sodium bicarbonate before breeding has also been claimed (14), and there is evidence that diet may effect the ratio (11).

Postnatal development.—Mice are born hairless, except for short vibrissae, and with eyes and ears shut. Sex can be distinguished at birth: males have the larger genital papilla, and there is a greater distance between this and the anus in males than in females. At nine days females show five pairs of conspicuous nipples, though these tend to be obscured in a few days by the lengthening fur. The external ears have opened by three days. A well developed coat is present at two weeks. At twelve to fourteen days a number of interesting changes occur. There is a break in the growth curve, the eyes open, the external ears commence a rapid growth, the first moult begins, the larger follicles in the ovary develop an antrum, there is an increase in muscular activity. At about the same time the young mice eat their first solid food.

Table 3

DATA INDICATING THE AGE AT WHICH MATURITY IS REACHED BY FEMALES IN TWO DIFFERENT STOCKS

Stock	Mean Age at First Estrus	Per Cent of Cases in Which First Mating Occurred at First Estrus	Per Cent of Pregnancies Resulting when First Mating Occurred at First Estrus	Per Cent of Pregnancies Resulting from Matings in Mature Mice
Albinos	39 days	75%	48%	80–90%
Colored	52 days	85%	47%	80–90%

In young mice the vagina is closed by a membrane. The age at opening varies considerably both within and between stocks. In one series of 100 mice the age at opening ranged from 28 to 49 days with the median at 35 days (45). The first estrus as indicated by cornification of the vagina occurs soon after vaginal introitus. In one set of observations the interval was 24 to 120 hours (96). However, estrus, in the sense of willingness to mate,

probably does not always occur at this time. Data on the occurrence of the first estrus and the first mating have been published by Mirskaia and Crew (95, 96) and are summarized in Table 3.

As this table shows, the time of the first fertile mating varies greatly. Commonly it occurs at seven to ten weeks. Thirty-nine days is exceptionally early. Maturity in males occurs at about the same time as in females, or perhaps somewhat later.

The useful breeding period of most inbred females terminates when they reach ten or twelve months of age, for though litters may continue to be produced after this, breeding is apt to be irregular and the litters small. Hybrid females usually give fair sized litters and breed regularly until sixteen or eighteen months of age. Males will usually breed several months longer than females of the same stock.

Occasionally mice live to three years of age or even a few months past this.

Ovogenesis.—The problem of the origin of the female germ cells has been the subject of extensive study. The following description is based on the most important recent papers dealing with ovogenesis in mice and rats and does not present all the conflicting viewpoints found in much of the older and some of the more recent literature. All statements are based on work with the mouse unless otherwise specified. Investigations in this field have been ably reviewed by Heys (67) and Pincus (108).

Beginning at about nine (18) to eleven (72) days post coitus, the gonads of mouse embryos contain so-called primordial or primitive germ cells, characterized by their large size and by the clear appearance of the cytoplasm. These are present in both male and female gonads which at this early stage are indistinguishable. At this same time or slightly later, cells closely similar in appearance may be seen in tissues adjoining the gonads (18), a fact that has led to extensive speculation as to their place of origin and possible migrations. The view, at one time commonly held, that they migrate into the ovary and there give rise to the germ cells is not supported by recent evidence. The young primordial ova show numerous mitoses, though these soon cease. Proliferation of ova from the germinal epithelium, however, continues. By the twelfth to fourteenth day post coitus the nuclei of the oldest ova enter on the complex series of stages characteristic of the meiotic prophase, all of them reaching at least the pachytene stage by the time of birth (18, 25). Slightly before birth some of the primordial ova have begun to be surrounded by follicle cells, and by three (55, 70) to six (18) days post partum all the oocytes in the ovary proper have a follicular epithelium. The

number of these primordial ova is enormous. Arai (8) estimates that there is a total of approximately 35,100 in the two ovaries of a new born rat.

By birth, or shortly thereafter, another process has made its appearance; namely, the degeneration of ova. This is very evident in the ovaries of rats sixteen hours old and apparently reaches its height during the second and third day post partum (25). Some follicles continue to grow, but degeneration also continues, so that despite the production of new ova described below, the total number of ova in both ovaries of twenty-three day old rats is reduced to an average figure of about 11,000 (8).

The proliferation of ova by the germinal epithelium continues after birth (Fig. 32). There is some evidence that the process temporarily ceases or at

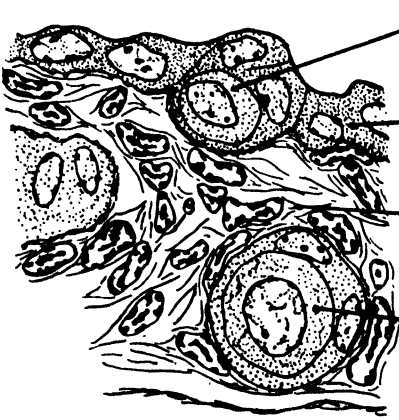


FIG. 32.—The formation of ova from the germinal epithelium in a 45 day old rat. (*After Hargitt.*)

least is somewhat retarded from birth until several days thereafter (25, 70), but this has also been denied (130). In any case, active proliferation is in progress at six or seven days post partum. The young ova are distinguishable from the other cells in the germinal epithelium by their larger size, clear cytoplasm, spherical and intensely staining nuclei, and by the fact that they often occur in pairs. At eight days post partum ova may be seen separated from the epithelium and in the process of passing through the thin tunica albuginea toward the underlying stroma (25). At twelve to fifteen days some of the follicles first acquire a small antrum (18, 44, 70). Accompanying this (fifteenth day) the diameters of the larger follicles show a sudden and pronounced rise to a size almost equal to that of the follicle at the occurrence of the first estrus (44). In rat ovaries, according to Lane (77), the percent-

age of follicles containing an antrum, as compared with the total number of follicles having at least two layers of follicular cells, is 11% at fifteen days. This figure rises to 39% at thirty-seven days, falls to 29% at fifty days, and then ascends sharply until it reaches 50% at sixty-six days when ovulation occurs. Hargitt (62) likewise has noted a decrease in the number of large follicles in rat ovaries two to three weeks before the first ovulation, and finds this to be due to an increased rate of degeneration of such follicles at this time. Ovulation in his animals occurred at about 45 days, and the ovaries at 29–32 days showed a drop in the number of large follicles. In the case of mice, also, a reduction in the number of large follicles in the ovaries of animals 28 days old, as compared with the number at 21 days, has been noted (18).

According to a recent study with rats (124), ovogenesis between birth and maturity is cyclic, with maxima occurring approximately every ten days. In this investigation, as in others described above, the first maximum was found to occur at six or seven days post partum. Other maxima occurred at approximately ten day intervals until the onset of the normal estrous rhythm. Follicular atresia during this period was found also to be cyclic with about ten days between peaks. How this prepuberal rhythm of ovogenesis and atresia is related to the prepuberal fluctuations in the proportions of large follicles described by other authors is not yet clear.

The process of ovogenesis continues, though somewhat more slowly, until fecundity is lost in old age. During maturity it shows fluctuations that coincide with the estrous cycle (see p. 74). The process is less conspicuous in older mice because the newly formed ova do not attain such large size while still in the germinal epithelium and hence are more easily confused with epithelial cells. Some authors have disputed the continued production of ova by the germinal epithelium during maturity, but recent work quite definitely confirms its occurrence (4, 25).

Coincident with the occurrence of ovogenesis, continued ovular degeneration is likewise going on. As a result there is a more or less steady reduction in the number of ova present in the ovaries. Counts by Arai (8) in the rat show a total of approximately 35,100 ova in both ovaries at birth, 11-10,000 at 23 days and 63 days, 6,600 at 70 days, 2,000 at 31 months. Except for the period from 23 to 63 days, ovogenesis is not sufficiently rapid to replace the ova lost through ovular degeneration and normal ovulation (For the details of the degenerative changes in atretic ova and follicles see p. 154.)

In addition to abnormal ova due to degenerative changes, polyovular follicles and polynuclear ova, probably not due to degeneration, have been

described as occurring occasionally in ovaries of both mouse and rat (43, 78).

The earlier workers in this field were puzzled by the fact that while ova formed before birth showed all the stages typical of meiotic prophase in the male, these stages were not found in ova formed after birth. This problem has been at least partly resolved by Swezy (130) in a study of the ovaries of female rats from before birth to maturity. At five days post partum the typical miotic prophase stages are, in fact, present. Deutobranch, leptotene, synaptene, pachytene and diplotene nuclei can all be distinguished. From then on the process is steadily modified and probably shortened. At twenty days deutobranch nuclei and nuclei showing modified pachynema stages may be seen. In the adult most of the different maturation phases are lost altogether, or at least are not cytologically distinguishable. Crew and Koller (32), however, have figured clear chiasmata in diplotene chromosomes in ova of mature female mice. This is excellent evidence that synapsis (and crossing-over) has occurred, even though the stage at which it occurs is difficult to see. Hence, however much the maturation stages may be modified and telescoped in the developing ova of adult mice and rats, there is little reason to doubt that they include the steps necessary for accomplishing the pairing and crossing-over of the chromosomes required by genetic evidence.

While the concomitant occurrence of ovogenesis and ovular degeneration at all ages until senility is reached seems well-established, the rate of turnover, and the consequent length of life of the individual ovum, remains somewhat uncertain. The view of early investigators that ova formed in the embryo are functional in the adult has been largely abandoned, and some writers have gone to the other extreme, maintaining that "individual follicles have a functional life span of only a day or two, in all cases less than the length of the estrous cycle" (49). Lane and Davis (79), as a result of studies of mitotic activity and volume changes in rat follicles, take an intermediate position. They write as follows: "Follicles less than 200 μ in diameter are inactive mitotically and are thought to be physiologically quiescent." In the adult, follicles of this size or smaller "represent a reserve from which are drawn succeeding crops of follicles for maturation at succeeding estrous periods. This follicle reserve will develop or be maintained without the assistance of the pituitary, but for the production of follicles larger than 200 to 300 μ, pituitary assistance is required. . . . Between 200 and 300 μ diameter, the follicle in any stage of the cycle shows mitotic activity in the granulosa and theca which is slightly augmented. These follicles are thought to be on the way to maturation or atretic degeneration. . . . It

seems significant that the follicles in the size range of 401 to 500 μ should exhibit the maximum activity in the granulosa. It is possible that these constitute the group which will ovulate at the succeeding estrous period. Numerically there are 6 of these follicles in the average metestrus ovary. Allowing for atresia this number could easily produce the 3 to 5 ovulating follicles which are present in each ovary at estrous."

A rough calculation of the length of life of the average follicle is possible from published data. In two experiments (98, 125) female mice were irradiated with x-rays and the condition of the ovaries determined by breeding tests or by histological study. These experiments show that fertile matings may be obtained from females irradiated with a dose of 260 r for a period not exceeding 28 days following treatment. After this they become completely sterile, presumably because no new ova are proliferated by the germinal epithelium (125). That irradiation causes early cessation of ovogenesis is shown by the fact that small or primary follicles are absent in ovaries of irradiated mice (150 r) killed 2 days after treatment. At 21 days only a few normal follicles of the older types are present, the gland being mainly composed of degenerating remnants. At 43 days there is a total absence of all follicular structure (98). These two experiments show that, at least in irradiated ovaries, ova can survive for only about 28 days following their proliferation by the germinal epithelium.

Further evidence as to the rate of development of ova is provided by experiments in which rat ovaries were ligated, so that degeneration resulted from loss of blood supply, followed by regeneration when the circulation was re-established (26). At 8 or 10 days degeneration is usually complete except for small peripheral regions. At 12 days small ova may be seen recently differentiated from the germinal epithelium. At 21 days some medium sized follicles are present. At 30–34 days recovery is practically complete, and the ovary contains fully developed Graafian follicles. The interval from the first appearance of new ova to their final full development is thus 18 to 22 days. This may be taken as the minimum time required for the complete development of ovum and follicle. It is, of course, possible that ova and follicles in normal ovaries develop at different rates and survive for shorter or longer periods than do ova and follicles in ovaries under the experimental conditions described above. The available evidence, however, points to 18 to 28 days as a reasonable estimate of the time taken for the mouse or rat ovum to mature following its separation from the germinal epithelium. A quite different line of evidence is provided by experiments with ovaries of embryonic or new born rats and mice grown *in vitro*. Under

these conditions, survival of primitive germ cells for at least 115 days has been recorded (91).

The high mortality among ova under normal conditions can be appreciated from figures presented by Allen (4). An average of 800 to 1000 ova are differentiated in the two ovaries of a female mouse at each estrous period, while only about 9 ova of an earlier generation mature. The percentage of survival is thus about 1%.

Brambell (19) has made an interesting study of the growth of oocyte and follicle, finding the relation shown in Fig. 33. It will be seen that at first both oocyte and follicle increase in size, the relation between the two being

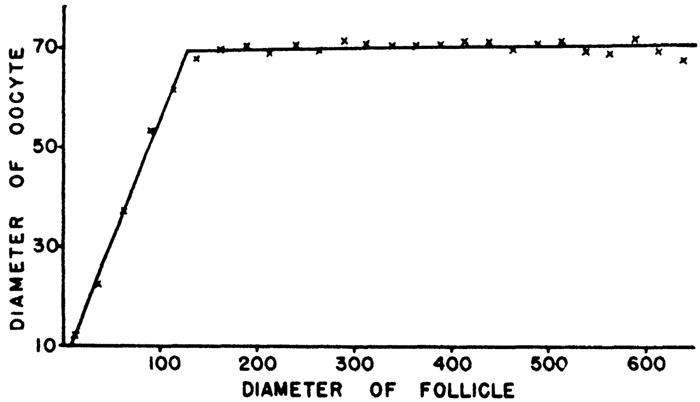


FIG. 33.—Graph showing the relation between oocyte size and follicle size. (*From Brambell* 1928.)

linear. When the oocyte attains a diameter of approximately 70 μ, and the follicle a diameter of 125 μ, the former stops growing while the growth of the follicle continues, so that the average follicle diameter at ovulation is 550 μ. The antrum first appears as an irregular fluid-filled cleft in the middle of the follicular cells on one side of the oocyte in follicles about 200 μ in diameter.

Ovarian regeneration.—As a result of reports in the medical literature of conception following complete double ovariectomy, there have been a number of experiments with mice and rats to determine whether or not there is any ovarian regeneration following removal of both ovaries. While the results have been conflicting, the most recent experiments seem to indicate that regeneration does not occur if the removal is complete (66, 107). If regeneration does occur it is probably the result of a small piece of ovarian tissue having been left. Regenerated ovaries contain follicles and may be fully functional (37, 105).

Butcher (26) has described regeneration of rat ovaries following ligation. At eight or ten days the ligated ovaries consisted almost entirely of degenerate tissue. In the periphery of the ovary, particularly in the region where a cavity had persisted between the gonad and capsule, small or primary follicles were found, but in no case did the number encountered in one ovary exceed twenty. Recovery was rapid, the ovary being practically normal at thirty to thirty-four days. These cases of regeneration in the adult rat and mouse are excellent evidence that ovogenesis can occur in sexually mature animals.

In view of the extent of ovarian regeneration following partial removal it is noteworthy that the ovaries of mice sterilized with x-rays, either at birth or later in life, never regenerate any germ cells although they remain functional in regulating the estrous cycle (22, 98).

Robertson (111) has described the successful transplantation of ovaries between mice of the same inbred strain.

The estrous cycle.—Our present knowledge of the estrous cycle in rodents dates from the discovery of Stockard and Papanicolaou that the cellular contents of the vagina undergo cyclical changes and that by observing these changes in vaginal smears the successive stages of the estrous cycle can be accurately followed and the time of heat determined. The estrous cycle of the mouse has been studied by Allen (3), Rietschel (110), Clauberg (29), and others. Long and Evans (83) have published a very thorough study of the cycle in the rat. The following description is based on Allen's studies except as otherwise noted.

Divisions of the estrous cycle.—The estrous cycle of the mouse and rat is conveniently divided into 5 stages, namely, proestrus, estrus or heat, metestrus-1, metestrus-2, diestrus. The first two are anabolic stages during which active growth is in progress in various parts of the genital tract. They culminate in ovulation and, where mating occurs, in fertilization. The second two, metestrus-1 and metestrus-2, are catabolic stages characterized by degenerative changes in the genital tract. The last, diestrus, is a period of quiescence or slow growth. The characteristics of each stage are summarized in Table 4.

External signs of estrus.—There is a tendency at proestrus and estrus for the vulva to show swelling and congestion, and for the vaginal orifice to gape, but these appearances are so variable as to be unreliable signs of heat. The onset of heat in the rat can be accurately determined by the "copulatory response" (65, 137).

Table 4

SCHEMATIC OUTLINE OF CHANGES IN THE REPRODUCTIVE ORGANS OF THE MOUSE DURING THE ESTROUS CYCLE

Stage	Smear*	Histology of the Vaginal Epithelium	Uterus	Ovary and Oviduct
Proestrus	E to EC or ECL to EC	Many cell layers (10–13). Outer 4–5 nucleated, stain lightly with eosin. Under these, granulosa layer showing increasing cornification. Active mitoses. Few leukocytes.	Increasing hyperemia and distension. Active mitoses in epithelium, few leukocytes.	Follicles large and distended with considerable liquor folliculi. Few mitoses in germinal epithelium and in follicular cells.
Estrus	EC to C^{+}	Superficial nucleated layer lost. Cornified layer now superficial. About 12 layers of nucleated cells under this. Mitoses decreasing. Leukocytes absent.	Distension and mitotic activity reach maximum during estrus, and then decrease. No leukocytes.	Ovulation occurs followed by distension of the upper end of oviduct. Active mitoses in germinal epithelium and in follicular cells.
Metestrus-1	C^{++}	Cornified layer delaminated. Leukocytes begin to appear under epithelium.	Distension decreased. Leukocytes begin to penetrate epithelium.	Early corpora lutea present. Eggs in oviduct. Many follicles undergoing atresia.
Metestrus-2	$C^{++}EL^{++}$	4–7 layers of epithelial cells, with very many leukocytes in outer layers.	Walls collapsed. Epithelium shows degeneration. Mitoses rare. Leukocytes numerous.	Growing corpora lutea. Eggs in oviduct. Few mitoses in germinal epithelium and in follicular cells.
Diestrus	EL, more or less mucus	4–7 layers of epithelial cells, with leukocytes in outer layers. Growth commences towards end of diestrus.	Anaemic, walls collapsed. Epithelium healthy but contains many leukocytes. Some secretion by uterine glands.	Follicles begin rapid growth towards end of period.

* E = epithelial cells, C = cornified cells, L = leukocytes, $^{+}$ indicates many cells, $^{++}$ indicates very many cells. The smears given are typical; there is considerable variation.

Vaginal smears.—Three methods are in common use for taking vaginal smears. *1. Pipette or lavage method.* A pipette drawn to a rather fine point and containing a few drops of water is inserted into the vagina, the water ejected and immediately sucked in again. The water with its cellular contents can then be transferred to a slide for examination. *2. Spatula or curette method.* Some of the cell contents of the vagina can be removed by

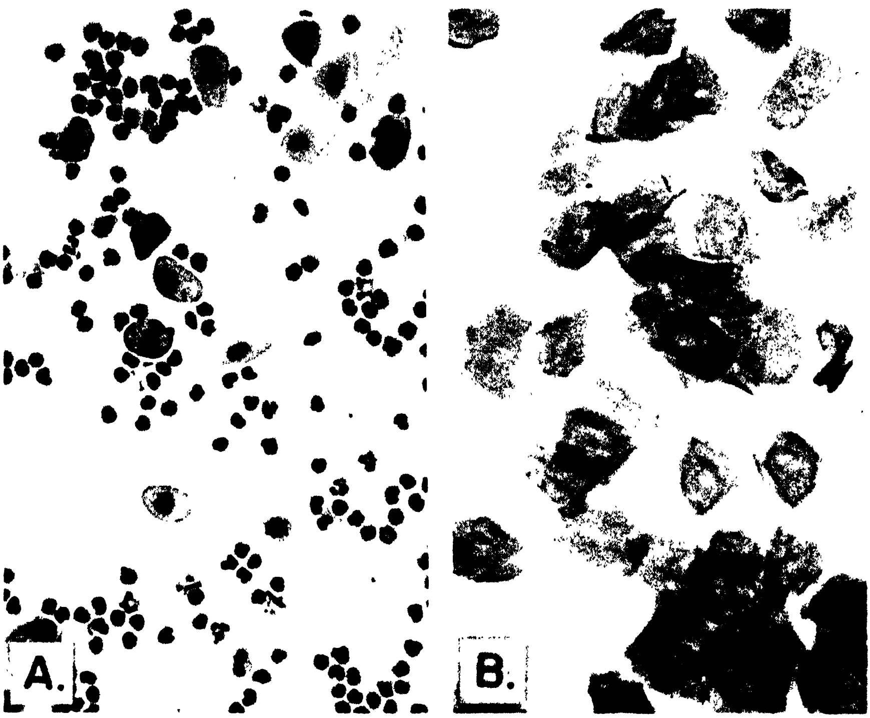


FIG. 34.—Photographs of vaginal smears stained with haematoxylin-eosin. A. Diestrus. B. Late estrus. (×300.)

means of a spatula or, preferably, a fine curette. The cells are transferred to a drop of water on a slide by tapping the curette on the slide. *3. Cotton swab method.* Cells can be removed with a fine, moist, cotton swab on the end of a toothpick. It has been shown that frequent smearing with cotton swabs will produce cornification of the vagina in spayed rats and mice, hence leading to a smear that indicates estrus though in animals in which true estrus cannot occur (133). The cycle in normal animals may likewise be disturbed by this method of smearing (40) which is, therefore, not to be recommended. The lavage method is less upsetting (40, 41). The curette method is probably also satisfactory though it has been noted that frequent

smearing with a spatula tends to disturb the regularity of the cycle (113, 133). The addition of a small amount of methylene blue to the water used gives a very satisfactory stain. With this stain smears can be examined at once without waiting for the water to dry.

Three types of cells are found in vaginal smears. *1. Leukocytes* (Fig. 34A). In unstained preparations these appear at first as small, round, highly refractive cells, but they swell rapidly in water with resulting rupture of the cell wall. In preparations stained with methylene blue the polymorphic nucleus takes a strong stain. *2. Cornified cells* (Fig. 34B). These

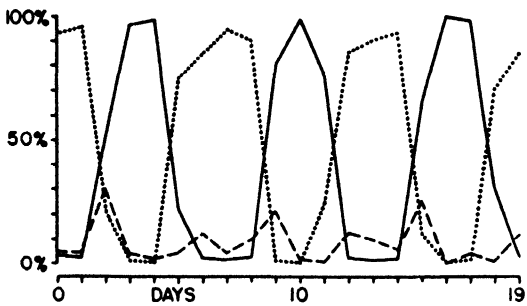


FIG. 35.—Graph showing the percentages of each of the three types of cells in the vaginal smear of a mouse during the normal estrous cycle. Smears taken daily. ——— cornified cells, ------ nucleated epithelial cells, ... leukocytes. (*Voss* 1930.)

are the largest cells in the smear. They are flattened, angular in outline, quite regular in size, and lack nuclei. *3. Nucleated epithelial cells* (Fig. 34A). The typical epithelial cell is round, oval or polygonal, with clear cytoplasm and a centrally placed nucleus that takes a strong methylene blue stain. A number of variations occur. As estrus approaches the smear may contain epithelial cells with dark staining cytoplasm and karyolytic nuclei. The cytoplasm may contain droplets (of mucus?). A highly modified mucus-secreting type also occurs (110). This characteristically is goblet-shaped with the nucleus at the apex. The presence of mucus can be proved by the use of appropriate stains. Cells intermediate between cornified cells and nucleated epithelial cells occasionally occur.

More or less mucus may occur in the smear. Different accounts differ greatly as to the amount normally present. It is possible that smearing or other forms of irritation increase the amount. In adult ovariectomized rats, mucification is produced by the combined administration of oestrone and progesterone (115). During the latter two thirds of pregnancy in the rat the vaginal mucosa actively secretes mucus (54, 75).

The cyclical changes in the cell contents of the smear are shown in Figs. 35 and 36, taken from Voss (132). Unpublished data obtained at the Jackson Laboratory indicate striking strain differences. However, the cycles shown in Voss's figures may be taken as fairly typical. The smears in terms of which the various stages of the cycle are defined are indicated in Table 4.

Of particular interest is the smear characteristic of estrus or heat. The complete or almost complete absence of leukocytes from the smear and the presence of cornified cells, in moderate numbers and not clumped, are the usual criteria of estrus. In one study (126) with MacDowell-Bagg albino mice it was found that the smear at the onset of heat, as determined by

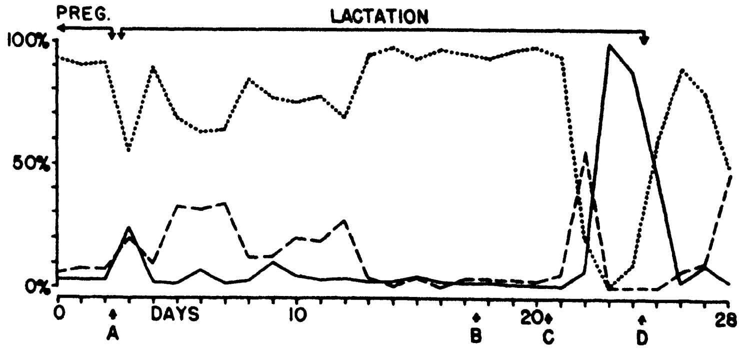


FIG. 36.—Graph showing the percentages of the three types of cells in the vaginal smear of a mouse during a postpartum estrus, lactation, and the normal estrus following lactation. Smears taken daily. ——— cornified cells, ------ nucleated epithelial cells, ... leukocytes. (A) litter of four born and the beginning of lactation, (B) two young weaned, (C) one more young weaned, (D) last young weaned. Note the incomplete cornification at the post partum estrus and the occurrence of a normal estrus while one young is still nursing. (*Voss* 1930.)

willingness to mate, still contained 5 to 75% of epithelial cells. The smear marking the termination of estrus has not been so accurately determined, but the presence of clumps or sheets of cornified cells is usually regarded as marking the onset of metestrus. A typical late estrus or early metestrus smear is shown in Fig. 34B.

The vagina.—No part of the genital tract undergoes more striking histological changes during the estrous cycle than the epithelium of the vagina. The successive stages are shown in Figs. 37 and 38 and summarized in Table 4.

In proestrus the epithelium consists of three layers (Fig. 37A). The outer layer is composed of epithelial cells sometimes more or less filled with

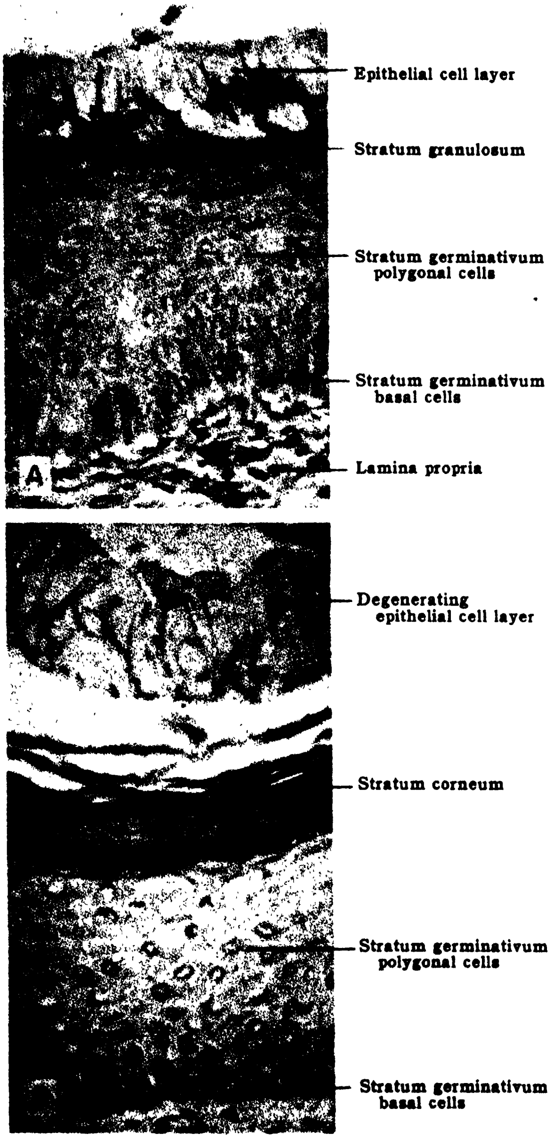


FIG. 37.—Photographs of vaginal epithelium of a mouse in different stages of the estrous cycle. A. Proestrus. B. Estrus. (*From Clauberg.*)

mucus and with nuclei showing signs of pycnosis. Below this is the stratum granulosum which, with the approach of estrus, becomes the stratum corneum. Third is the stratum germinativum, some seven cell layers in

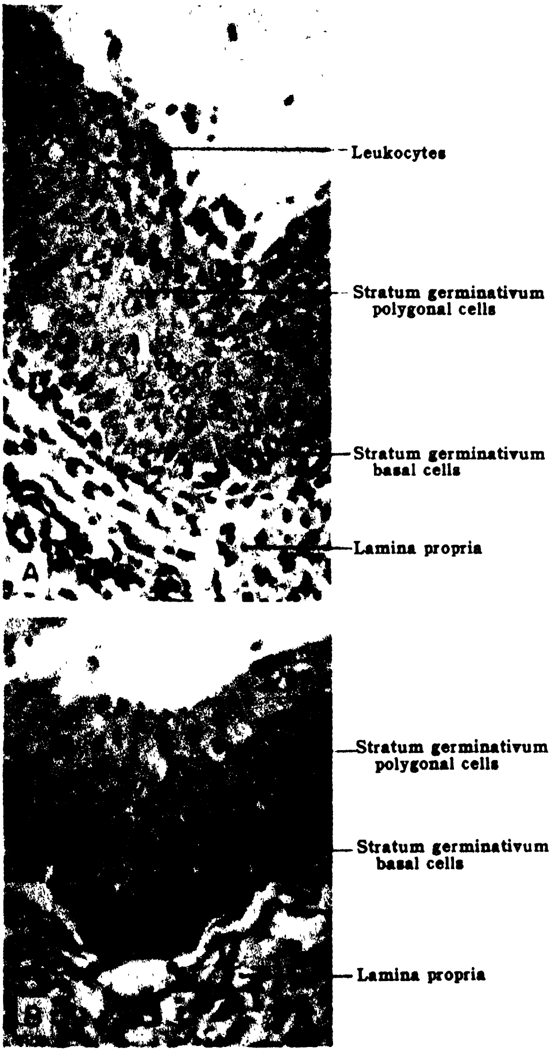


FIG. 38.—Photographs of vaginal epithelium of a mouse in different stages of the estrous cycle. A. Metestrus-2. B. Diestrus. (*From Clauberg.*)

thickness. During proestrus and early estrus the cells of the outer layer are delaminated into the vagina, producing the characteristic nucleated cell smear. The degree of delamination is not uniform in all parts of the vagina, so that prior to the onset of estrus the cornified layer may be fully

exposed in some regions, not at all in others. During late proestrus and throughout estrus cells are delaminated from the cornified layer (Fig. 37B). The onset of metestrus-1 is characterized by the peeling off of the whole layer, with an accompanying rise in the cornified cell count in the smear. During metestrus-2 there is a rise in the nucleated cell count (Fig. 35), indicating that in the last stages of the delamination process some of the superficial layers of the stratum germinativum are included. The superficial layers of the stratum germinativum, meantime, have become heavily infiltrated with leukocytes (Fig. 38A) which also appear abundantly in the smear at this time. As a result of the delamination of the superficial layers, the vaginal epithelium at diestrus contains only one layer, the stratum germinativum, some three to seven cell layers in thickness (Fig. 38B). Late in diestrus active growth begins in the stratum germinativum, and by proestrus a stratum granulosum has formed several cell layers below the surface, thus completing the cycle.

The uterus.—The uterus, like the vagina, undergoes a series of anabolic and catabolic changes during the estrous cycle, but they are relatively much less striking (Table 4 and Fig. 39). In proestrus and early estrus the uterus shows marked hyperemia and is distended with fluid secreted by the uterine glands. The distension starts to diminish in late estrus and in diestrus the uterine wall is collapsed and anaemic. It has been reported that in the rat the loss of some of this fluid is due to discharge into the vagina (83). The uterine epithelium has been described as low columnar in proestrus, with a distinct basement membrane, as high columnar in estrus (3, 29). The increase in height is not marked, however, and in an experiment with the rat a reverse change was noted accompanying the increasing distension of the uterus (7). In metestrus-1 degenerative processes become apparent. The basement membrane fades into a pink-staining band which includes the basal sides of the epithelial cells and the superficial stroma. The epithelium loses its definite organization and shows vacuolar degeneration. Leukocytes appear in the region of the basement membrane. In metestrum-2 the degeneration of the epithelium is further advanced, so that almost all the epithelial cells are lost (110). Cell walls at this stage are no longer recognizable and leukocytes are numerous. The uterine glands show minimum activity. The onset of diestrus is marked by the beginning of regenerative processes.

The oviducts.—In the case of most mammals the oviducts show hyperemia at estrus (6, p. 668) and the same is probably true of mice, though the condition seems not to have been specifically noted. There is none of the

periodic leukocytosis so marked in the rest of the genital tract. Cyclical changes in certain non-ciliated cells in the epithelium of the ampulla have been both described and denied (2, 3, 48, 110). These cells protrude into the lumen of the tube in an unusual fashion, and there is some evidence that the protrusion shows cyclic changes. For some hours following ovulation the upper part of the oviduct is distended with fluid.

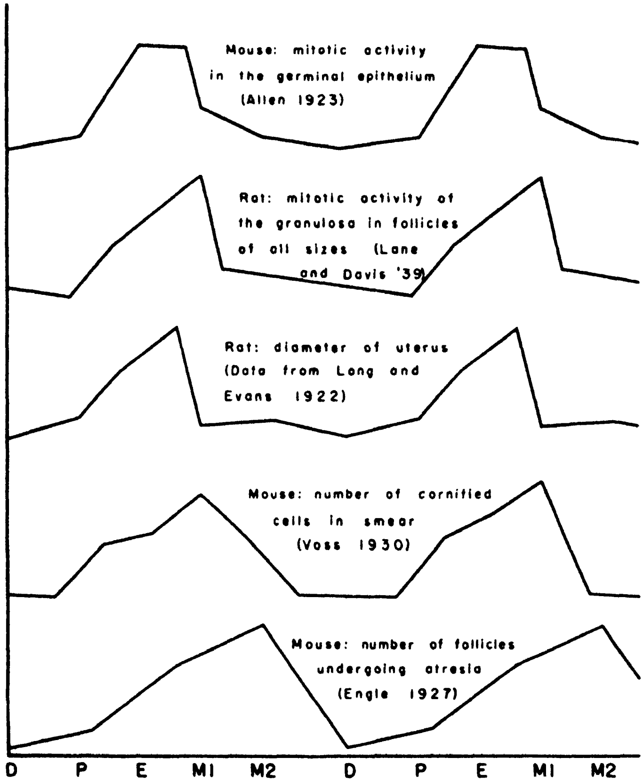


FIG. 39.—Graph showing various cyclic phenomena which accompany the estrous cycle. The curves have been adjusted to make corresponding points of the cycle correspond as nearly as possible. D = diestrus, P = proestrus, E = estrus, M1 = metestrus-1, M2 = metestrus-2.

The ovary.—Cyclic changes are pronounced in the ovary (Fig. 39 and Tables 4 and 5). A conspicuous feature is the rapid growth of a few of the follicles prior to ovulation. Lane and Davis (79) sectioned the ovaries of twenty rats, five in each of the four major divisions of the estrous cycle, and classified the follicles according to diameter. The results are shown in Table 5. It will be seen that by proestrus the follicles that will ovulate at estrus are fairly definitely set apart by their larger size. Brambell and

Parks (21) have made a study of the rate of growth of maturing follicles in unmated mice. They find that the follicles which will ovulate at the following estrus are, on the average, only 380 μ in diameter at the beginning of the estrous cycle. The follicles reach a maximum size of, on an average, 550 μ in diameter immediately before rupturing. Most of this growth, according to their study, occurs in the last 48 hours, during which period the follicles increase 45% in diameter. Secretion of secondary liquor folliculi, which at this time begins to replace the less fluid primary liquor folliculi, may play a considerable role in the increase in size (6, p. 458; 131). Just prior to ovulation the follicles bulge conspicuously from the surface of the ovary.

Table 5

AVERAGE DISTRIBUTION OF FOLLICLES ACCORDING TO SIZE THROUGHOUT THE ESTROUS CYCLE IN THE RAT (FROM LANE AND DAVIS)

Follicle Diameter (in Micra)	Diestrus		Proestrus		Estrus		Metestrus	
	No.	%*	No.	%	No.	%	No.	%
35–100	130	61.3	72	53.3	63	50.0	89	56.4
101–200	55	26.0	43	31.8	41	32.5	48	30.4
201–300	12	5.7	11	8.1	11	8.7	10	6.3
301–400	7	3.3	2	1.5	5	3.9	6	3.8
401–500	4	1.9	1	0.7	1	0.8	5	3.1
501–600	3	1.4	3	2.2	2	1.6	0	
601–700	1	0.4	3	2.2	3	2.5	0	
Average total	212				126		158	

* The percentage of the total follicle content which falls in a given size range.

Several studies (4, 5, 79) have shown that the mitotic activity in the ovary is cyclic, reaching a peak at estrus or metestrus-1 (Fig. 39). Since mitosis in the vaginal epithelium reaches a peak in proestrus or early estrus (3, 83), it appears that the ovary responds to the estrus stimulus more slowly than the vagina. Follicular atresia, like follicular growth, is cyclic, reaching a peak in metestrus-2 (Fig. 39).

In metestrus-1 newly formed corpora lutea are present. Since corpora lutea in unmated mice persist for two, three or four cycles before disappear-

ing, numerous old corpora lutea are also present in females which have been unmated for several previous cycles.

The mammary glands.—The mammary glands show cyclic growth and regression, though the changes are slight compared to those occurring during pregnancy (30, 82). In proestrus buds appear on the ducts particularly around the periphery of each gland, and large blunt projections appear on the main ducts near the nipples. In estrus the mammary ducts become dilated, and the buds formed during proestrus prolongate. Metestrus-1 introduces regressive changes and by the end of metestrus-2 the ducts are decreased in width and the duct endings collapsed. In diestrus the mammary gland consists of a very open network of narrow, thread-like ducts with comparatively few branches, the branches themselves being simple.

Other concomitants of estrus.—It has been noted that in the rat bodily activity, as measured by the number of hourly revolutions of a rotating drum placed in the cage, increases during estrus (65, 121, 135). A loss in weight at estrus has been described in mice (1), but the weight cycle was not regular except in mice with a very long estrous cycle (13–14 days), and it does not seem to occur in rats (122). A cyclic change in the electrical potential between the vagina and the symphysis pubis has been described in rats (112). There is a marked peak in potential in late estrus, with an abrupt fall when estrus terminates. A minor peak occurs about two days before estrus.

The postpartum estrus.—An estrus occurs in mice and rats within about 20 hours of parturition. The range for mice in the interval between parturition and the following ovulation has been found to be about 14 to 28 hours (84). The cornification of the vagina is not complete at this estrus, and the cornified cell content of the smear never reaches 100% (Fig. 36). Fertile matings are less often obtained during this period than during the course of the normal cycle. There is less fluid in the uterus than during a normal estrus (93).

The time relations of the cycle.—In the mouse the onset of heat usually occurs in the night, most commonly between 10 P.M. and 1 A.M. Occasionally it occurs between 1 and 7 A.M., in only rare instances during the day (81, 126). Similar results have been obtained with the rat except that the modal hour for the onset of estrus is several hours earlier, heat usually beginning between 4 and 10 P.M. (12, 31, 121). The onset of heat may be made to occur in the daytime in either mice or rats by keeping them in a room that is dark in the daytime, lighted at night (23, 52, 65, 126).

Observation of 608 heat periods in the rat showed an average duration of 13.7 hours, with a range of 1 to 28 hours (12). Periods that start early in the evening tend to run somewhat longer than ones that start later (31). The duration has not been so accurately determined in mice, but is probably much the same as in the rat. One estimate has placed it at about 12 hours (126).

In some cases what is commonly regarded as the estrous smear may last for long periods. Allen (3) found that "as diagnosed by the smear method," estrus usually lasts 1 or 2 days, but that unbroken estrous smears may continue for 9 days, and that 4 days of "heat" are not uncommon. These cases of long continued estrous smear may be the result of the irritation due to smearing (133), or they may occur normally in certain strains. The ce strain commonly shows long intervals of cornification (Ossen, unpublished data, 50). In any case they cannot be taken to indicate a long duration of actual heat without further evidence.

The modal length of the complete cycle is commonly 4 days in rats (12), 5 days in mice. Parkes (101) found the following distribution for 1000 cycles in unmated mice: 2 days, .4%; 3 days, 2.9%; 4 days, 15.8%; 5 days, 29.3%; 6 days, 21.8%; 7 days, 12.2%; 8 days, 6%; 9 days, 3.1%; 10 days or more up to 28 days, 8.5%. There seems to be a tendency for the length of the cycle to increase with the age of the female (120). There are marked strain differences in the length of the cycle (1, 4, 24). Strain differences are even more pronounced in the matter of the individual stages of the cycle as indicated by the smear. The diestrus interval is commonly the longest interval, and also the most variable. The daily changes in the smear throughout three typical cycles are shown in Fig. 35. In these particular cycles the approximate lengths of the different stages are: proestrus, 1 day; estrus, ½ day; metestrus-1, 1 day; metestrus-2, 1 day; diestrus, 2½ days.

In the rat, low temperature has been shown to lengthen the cycle (16, 80).

Ovulation.—Ovulation occurs spontaneously during estrus in both mice and rats, whether mated or unmated. Different accounts differ considerably as to the time of ovulation in relation to the onset of estrus, a fact perhaps due in part to the existence of significant strain differences. Ovulation in mice has been said to occur both at or near the beginning of estrus (21, 81, 126), and at or near the end of estrus (3, 131). In a recent study (126) with MacDowell-Bagg albino mice it was found that ovulation usually occurred between 12 M. and 2 or 3 A.M., but in one case at least as early as 11:30 P.M. and in another at least as late as 4:40 A.M. Since

mating most commonly occurred between 10 P.M. and 1 A.M., the average interval between the onset of estrus and ovulation for the MacDowell-Bagg albino strain at least is probably about 2 hours. The interval was found to be quite variable, however, being certainly less than 1 hour in one case and certainly more than 3 hours and 45 minutes in another. Ovulation within 15 minutes of mating has been noted (81). Extensive data for the rat (17) indicate that ovulation in the Wistar strain commonly occurs some 9 hours after the onset of estrus, but may occur at least as early as 7½ hours and at least as late as 12½ hours after the onset of estrus. It should be remembered that the onset of estrus occurs much earlier in the evening in this species than it does in the mouse.

The rupture of all the mature follicles in an ovary seems usually to be approximately synchronous (83), but there is evidence that an appreciable interval may separate the individual ovulations in some cases (3, 81, 126). Ovulation may not occur at every estrus, particularly in young virgin females (3, 131). Conversely, estrus may not always accompany ovulation (137). The mechanism of ovulation is not entirely understood, but there is evidence that a thinning of the wall at the outer surface of the follicle and an increase in internal fluid pressure both play a part (6, 131).

Immediately after ovulation the eggs are found in the upper part of the oviduct, presumably carried there by an outrush of follicular fluid at the time of follicle rupture. The beating of the cilia of the infundibulum may also help to carry them from the capsule into the oviduct. At the same time the upper part of the oviduct becomes distended with fluid, a condition easily seen in dissected animals under the microscope (83, 126, 127). As the distension is not present prior to ovulation, it is a reliable sign that ovulation has occurred (126). It has been stated that most, at least, of the fluid is not derived from the follicles, but rather is secreted by the tubes themselves (21, 83).

Maturation and fertilization.—Maturation and fertilization of the egg in the mouse and rat have been described by several authors (71, 74, 76, 84, 127). The following description is based on the work of Long and Mark (84) except as otherwise noted.

The whole maturation process requires not less than 4 nor more than 15 hours. At the onset of estrus the first maturation division is usually in progress (126). Usually this division is completed, the first polar body present, and the second maturation spindle already formed by the time ovulation occurs. Occasionally, however, the egg is in the stage of the first spindle or the first telophase at the time of ovulation, in which case

first polar body formation is completed very shortly thereafter. The polar body is quite large. Its future history is variable; it may degenerate while the egg is in the one cell stage, or persist as late as the morula stage. Occasionally it divides in two (81). Where mating occurs at the onset of estrus, sperm are probably usually present in the upper end of the tube at the time of ovulation (126). Each egg is surrounded by a zona pellucida and, outside this, a covering of cumulus cells. The cumulus cells are sticky, and all the eggs in one tube usually are massed into a clump. The sperm penetrate these coverings quite rapidly, partly dispersing the cumulus cells in the process, perhaps by enzyme action (108), and reach the vitellus in less than 2 hours (81). The penetration of the vitellus may be regarded, by definition, as the actual moment of fertilization. At the time it occurs the second maturation spindle is invariably present. In the absence of fertilization, the second polar body does not form; where fertilization occurs, second polar body formation ensues rapidly (71, 81, 84), and the processes of normal development are initiated.

Since estrus lasts for some 12 hours in mice and rats, mating may occur several hours after ovulation, the eggs meantime lying unfertilized in the oviducts. For several hours they retain their capacity for normal fertilization and development, but in a relatively short time degenerative processes make their appearance (13, 108).

The transport of sperm and eggs.—Sperm reach the upper end of the uterus in the rat almost at once after mating (53, 58, 64, 114). Throughout heat the uterus is distended with fluid, and transport of sperm to the mouth of the oviduct is accomplished not by the sperm's own motility but as a result of a churning action of the uterine wall acting on this fluid. Transport of sperm through the oviduct is somewhat slower, but Lewis and Wright (81) find that they may reach the ovarian end of the oviduct, where fertilization occurs, within 15 minutes of mating. The mechanism involved in this transport of the sperm toward the ovary, as also in the abovarian transport of the fertilized eggs, is somewhat obscure, though a churning action may again be involved in the sperm transport. The subject has been thoroughly discussed by Parker (106) and Hartman (in Allen, 6), and the interested reader is referred to these authorities.

The spermatozoa of the mouse retain their fertilizing ability in the oviduct for about 6 hours; their motility ceases only after 13½ hours. Their period of survival in the uterus is shorter than in the oviduct (93).

Pseudopregnancy.—Sterile matings in the mouse and rat induce a condition called pseudopregnancy, characterized by a delay of the next

estrous period. In mice the average interval between a sterile mating and the next estrus is 11 days (38, 100); in rats the average interval is 14.5 days and the range 7 to 19 days (122). It has been shown that pseudopregnancy can be induced in the rat by several forms of artificial stimulation. These include the brief insertion into the uterine cervix of a fine glass rod (83), electrical stimulation of the vagina (60, 119), and intense electrical stimulation through the head (63). Rats stimulated by the probe method while under ether anaesthesia show only ten per cent pseudopregnancies as against sixty-nine per cent for the controls (94). Spinal anesthesia completely prevents the induction of pseudopregnancy. In the rat, copulation without plug formation is a much less effective stimulus than copulation with plug formation, and the chance that pseudopregnancy will be induced seems to be still further increased if several completed matings each with plug formation are permitted (9).

Pseudopregnancy is accompanied by important changes in the uterus paralleling those that occur during the corresponding stages of pregnancy and serving to prepare the uterus for the implantation of embryos. Histologically, the changes in the rat and mouse uterus are not as striking as those occurring in the rabbit, but definite progressive changes in the epithelium and stroma have been noted (7). More significant than the histological changes is the capacity of the uterus during the early part of pseudopregnancy to respond to appropriate stimuli by local growth of the decidua, giving rise to swellings called deciduomata. Any slight local injury to the uterus will incite their formation; a common practice is to use a silk thread inserted through the uterine wall (83). In the pseudopregnant mouse, the maximum capacity for deciduomata formation following local injury of the uterus occurs about three days post coitum; by five days post coitum the sensitivity is almost lost (103). The sensitive period thus corresponds to the period of normal implantation.

The mammary gland undergoes development during pseudopregnancy. The changes parallel those of pregnancy for the first nine days following copulation. At the end of this period the pseudopregnant development of the mammary gland reaches its peak, and regression sets in (30).

The available evidence, though not conclusive, seems to indicate that the remarkable causal chain by which a stimulus applied to the uterine cervix prepares the uterus to receive the young embryo involves a nervous impulse from the cervix to the pituitary, an endocrine effect of the pituitary on the corpora lutea, and a second endocrine effect of the corpora on the uterus and mammary glands.

Corpora lutea.—Following ovulation, the ruptured follicles, and occasionally also large unruptured follicles (83) undergo changes which transform them into corpora lutea. During the first few days of its development each young corpus passes through characteristic stages from which an approximate though probably not very accurate estimate of its age is possible (38, 39, 128. See also p. 151). The subsequent history of the corpus depends on the sexual history of the animal. On the basis of this history, four types of corpora may be distinguished. The following description of these, except as otherwise noted, is based on the observations of Long and Evans (83) on corpora lutea in the rat.

1. Corpora lutea of ovulation are corpora formed during an ordinary estrous cycle where mating does not occur, or at a postpartum estrus if mating or lactation do not occur. Such corpora may persist with little obvious degeneration through two, three, or four cycles in the mouse (3), possibly longer in the rat, so that an ovary from a mouse which has run several uninterrupted cycles often contains as many as sixteen large, well defined corpora. The youngest set is distinguished not only by the morphological characteristics which set it apart for the first one or two days but also by the fact that it stains blue with hematoxylin (3). Older sets have a greater affinity for eosin. Perhaps a more critical test is a change in certain lipoid droplets which can be detected in the luteal cells following appropriate fixation. These are small and regular in size in young corpora, become larger and less regular in size with the onset of the next estrus. Long and Evans (83) believe that the functional life of the corpus has terminated by the time the changes in the droplets appear. Another test of age is provided by the fact that the lutein cells of old corpora stain more readily than those of young ones with the vital dye Dianil Blue 2R injected intraperitoneally.

2. Corpora lutea of pseudopregnancy are corpora lutea formed following a sterile mating. Such a mating induces a diestrous interval of some eight or ten days, and throughout this interval the lipoid droplets in the newly formed corpora retain the small, uniform size characteristic of young corpora of ovulation. Corpora of pseudopregnancy become more highly vascularized (39) and attain a slightly larger size than do corpora of ovulation. There is evidence that the prolonged diestrus following sterile mating is caused by a lengthened functional life in the corpora of pseudopregnancy.

3. Corpora lutea of pregnancy are corpora formed following a fertile mating. For the first few days these cannot be distinguished from corpora

of ovulation or pseudopregnancy; for the next few days their development parallels that of corpora of pseudopregnancy. On the eighth day, however (in the mouse, 38), they begin a period of rapid growth largely accomplished by an increase in cell size, and by the sixteenth day they are almost twice the diameter of corpora of ovulation. They are made more conspicuous by the fact that, during pregnancy, ovulation is suspended and no new corpora formed, while meantime older sets of corpora rapidly regress. With appropriate vital stains traces of the corpora of pregnancy may be detected for three or four months post partum in rat ovaries.

4. Corpora lutea of lactation are the corpora that develop in non-pregnant nursing mice from the follicles that ovulate at the first post partum estrus. These corpora are distinguished from all others by the particularly small size of the lipoid granules. Within twenty-four hours of the removal of a nursing litter, the granules show the characteristic increase in size indicative of cessation of function of the corpora. Corpora lutea of lactation attain a size somewhat larger than that of corpora lutea of ovulation or pseudopregnancy, but not equal to that of corpora lutea of pregnancy.

Lactation.—The normal duration of lactation in mice is about four weeks. Milk production rises for the first ten days and then gradually declines (46). In lactating mice a long period of diestrus occurs following the first post partum estrus. If the nursing litter is of normal size, this diestrous period, called the lactation interval, lasts from about 20 to 25 days (34, 38). It may be terminated by removal of the litter, this inducing estrus in two to four days. If the stimulus of suckling is maintained by replacing the growing litters from time to time by very young litters, an estrus occurs as usual at about three weeks, but future estrous periods are delayed, the intervening diestrous intervals being some twelve to seventeen days long (116). The stimulus of nursing can produce a marked effect on females who have not recently lactated. Thus when normally cyclic adult mice or rats are given on alternate days new litters of actively nursing young, marked development of the mammary glands occurs. Milk secretion may even be induced. The condition is accompanied by a lengthening of the diestrous interval to two or three weeks, and by the appearance of the capacity for deciduomata formation in the uterus. When the nursing stimulus is removed, normal estrous cycles commence within a few days (116, 117, 118). It may be noted in this connection that deciduomata formation may also be induced during normal lactation (83, 85). Mammary involution following removal of a litter may be retarded in mice by irritation of the nipples with turpentine applied twice daily (68).

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Chapter 3

HISTOLOGY

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INTRODUCTION

This chapter gives the histology of the organs rather than the tissues, presupposing a general knowledge of the latter subject. The nervous system and the special sense organs are omitted, and for these subjects the reader is referred to the excellent work of C. W. Ariens Kappers, G. Carl Huber and E. C. Crosby (58), "The Comparative Anatomy of the Nervous System of Vertebrates Including Man."

The sections which serve as illustrations for this and for Chapter 4 were fixed in a mixture of alcohol, formalin and acetic acid (70% alcohol 100 cc., formalin 10 cc., acetic acid 5 cc.) for 4–24 hours, changed into 80% alcohol, dehydrated in the usual way and imbedded in paraffin. This technique gives satisfactory results with mouse tissue and is used in our laboratory

routinely. *Hematoxylin and eosin* (H & E) stains were used, unless otherwise stated.

Circulatory System

The blood vessels.—The walls of the blood vessels are formed of three parts: the innermost part, the interna or intima; the middle part, the media; and the outer part, the adventitia or externa.

The interna of the arteries consists of the endothelial lining, composed of elongated flat cells with prominent oval nuclei, beneath which is a network of elastic fibers forming the internal elastic membrane. The media is wide and in the large arteries consists of alternating layers of circular smooth muscle fibers and elastic membranes. According to Löwenthal (in Jaffé, 56), in the aorta six to ten such layers are present, intermingled with fine collagenous fibers. In smaller arteries the media contains less elastic and more muscular elements. In the arterioles it consists of only a few individual muscle fibers. The adventitia is composed of loose connective tissue fibers and serves as a transition zone between the arteries and the surrounding tissue.

The capillaries are formed of elongated endothelial cells usually separated from the surrounding elements by a thin sheath of connective tissue. They connect the terminal arteries with veins.

In some organs the connection between arteries and veins is accomplished through irregular spaces, the sinusoids. The walls of the sinusoids unlike the capillaries do not have a continuous endothelial cell lining, but are lined by scattered phagocytic and non-phagocytic cells.

The intima of the veins consists of polygonal-shaped endothelial cells and connective tissue fibers intermingled with fine elastic fibers. The media is formed of smooth muscle fibers and is poorly delimited from the next layer. The media of the wall of pulmonary veins contains cardiac muscle. The adventitia is well developed and contains connective tissue fibers intermingled with some longitudinal smooth muscle fibers. The valves of veins are formed of a connective tissue membrane containing a network of elastic fibers. Both surfaces of the valve are covered by endothelium.

The walls of all of the larger blood vessels are supplied with blood by small vessels, the vasa vasorum. In general the walls of veins in relation to the diameter of the lumen are thinner than the walls of the arteries.

The heart.—The heart consists of four chambers, the left and right atria and ventricles. These chambers are lined by endothelial cells which rest on a very thin layer of connective tissue. These together form the endo-

cardium. The myocardium is composed of cardiac muscle fibers which are arranged in spiral sheets. The myocardium of the left ventricle is considerably thicker than the right. Both atria have very thin muscle walls. The outermost layer is the epicardium. It is composed of a thin layer of connective tissue, covered by a single layer of mesothelial cells. A transparent, serous membrane, the pericardium, encloses the heart and the proximal portions of the great vessels. The heart lies in the pericardial cavity.

The right atrium communicates with the right ventricle by the tricuspid valve; the left atrium with the left ventricle by the mitral or bicuspid valve. The valves are composed of folds of the endocardium. They are covered on both sides by endothelium and contain some connective tissue. They are attached by thin strands, the chordae tendineae, to the projections of the papillary muscles of the ventricular walls.

According to Ohmori (74), the atrio-ventricular conducting system as described by Tawara exists in the heart of the mouse.

Arterial blood leaves the left ventricle through the aorta. The opening is guarded by the semilunar aortic valve. From the right ventricle the pulmonary artery originates, guarded by the pulmonary valve, and carries blood to the lungs. From the lungs oxygenated blood is transported to the left atrium through the pulmonary veins. The superior and inferior venae cavae bring venous blood into the right atrium.

The coronary arteries which branch off from the root of the aorta supply the tissues of the heart with blood. Capillaries are numerous among the heart muscle fibers.

Lymphatic capillaries and vessels.—The walls of lymphatic capillaries are formed of a single layer of large, flat, polygonal, endothelial cells. The lumina are irregular; dilations and constrictions occur frequently. The capillaries form many branches, some of which end blindly while others anastomose. The lymphatic vessels have thicker walls consisting of, in addition to the endothelium, collagenous bundles, elastic fibers, and smooth muscle fibers. In the larger lymph vessels an intima, media and adventitia can be distinguished. The intima is formed of endothelium, and a thin layer of elastic fibers, the media of circularly arranged smooth muscle fibers, while the well developed adventitia is composed of collagenous and elastic fibers and smooth muscle bundles. The paired valves are similar to those of veins and consist of folds of the intima. In the largest lymphatic vessel, the thoracic duct, the division of the three parts of the wall is very indistinct. Below the endothelium the collagenous and elastic elements form an inner elastic membrane from which fibers project and mingle with the smooth

muscle fibers of the media. The components of the adventitia are similar to those of the media and they merge gradually into the surrounding tissues.

Blood.—The red blood corpuscles or erythrocytes of the mouse are similar in shape to those of other mammals. They are very flexible, circular, biconcave discs, capable of becoming cup-shaped when passing through fine capillaries. The corpuscles contain hemoglobin and have lost their nuclei. They have a diameter of 5.7 μ according to Kerti and Stengel (in Jaffé, 56). Stained with Wright's stain, some of the erythrocytes show marked polychromatophilia (about 10% according to Simonds, 87). Nucleated red blood cells are seldom present in the circulating blood.

Haam (in Jaffé, 56) states that the hemoglobin content of the mouse blood (based on the average of the observations of nine investigators) is 97% (Sahli).

The white blood corpuscles or leukocytes are true cells with a nucleus and cytoplasm. Among them the lymphocytes are the most numerous. They are somewhat larger than erythrocytes and have large, spherical, slightly indented, eccentric nuclei which almost completely fill the cells. In stained preparations the nucleus is very dark; the cytoplasm is homogeneous and slightly basophilic.

The monocytes are the largest cells in the circulating blood. They have eccentric, ovoid, bean-shaped, occasionally deeply indented nuclei which stain lightly. The cytoplasm is abundant, non-granular and slightly basophilic.

The granular leukocytes are somewhat larger than the lymphocytes. Great variations exist in the shape of their nuclei, which may be ring-shaped or show irregular constrictions and lobulations. According to the staining reaction of the granules present in the cytoplasm of these cells, they are divided in three groups: neutrophilic, eosinophilic, and basophilic polymorphonuclear leukocytes. The eosinophilic cytoplasmic granules stain readily, while the neutrophilic granules stain faintly. The basophilic cells are very rare; some investigators consider them absent. Simonds (87) gives their number as less than 1%.

The blood platelets are very small, blue staining, granular bodies similar to those present in the human blood. According to Klieneberger and Carl (in Jaffé, 56) their number varies between 157,000 to 620,000 (mean 284,810) per cu. mm.

To obtain blood for counts the tail vein and the ventricles of the heart are most often used. Table 1 gives the total erythrocyte and leukocyte count and the differential leukocyte count of several strains of mice maintained

Table 1

TOTAL ERYTHROCYTE AND LEUKOCYTE COUNT AND DIFFERENTIAL LEUKOCYTE COUNT OF ANIMALS BETWEEN 77–86 DAYS OF AGE

Strain	No. of Mice	Erythrocyte Count (Number per Cu. Mm.)		Leukocyte Count (Ventricle Blood) (Number per Cu. Mm.)		Differential Count in Per Cent (Peripheral Blood)			
		Mean	Range	Mean	Range	Lymphocytes	Neutrophils	Monocytes	Eosinophils
C57 brown	13 ♂♂ 4 ♀♀ 17	9,704,000 9,580,000 9,378,000	(8,656,000–12,352,000)	4,280 4,900 4,380	(2,840–9,220)	71	14	12	3.0
C57 black	6 ♂♂ 7 ♀♀ 13	9,292,000 8,527,400 9,038,000	(7,784,000–10,244,000)	4,990 4,260 4,600	(3,950–5,550)	66	22	11	0.6
MacDowell-Bagg albino	3 ♂♂ 8 ♀♀ 11	10,426,000 9,733,500 9,921,820	(8,768,000–11,136,000)	4,820 3,660 3,880	(2,520–6,660)	63	23	13	0.3
dba	10 ♂♂	11,234,000	(7,592,000–13,168,000)	5,220	(3,320–6,700)	64	25	11	0.4
C_3H	10 ♂♂ 5 ♀♀ 15	8,841,000 8,606,600 8,760,800	(7,280,000–11,296,000)	3,930 2,940 3,600	(2,000–6,300)	63	23	14	0.2
Leaden	8 ♂♂ 8 ♀♀ 16	11,096,750 10,214,000	(9,440,000–13,936,000) (7,312,000–13,088,000)	5,740 4,662	(1,660–10,480) (2,100–10,020)	79 80	9 8	8 9	3.0 4.0

in the R. B. Jackson Memorial Laboratory. (The counts are based on unpublished data of Dr. L. W. Law and Dr. W. E. Heston.) The peripheral blood obtained from the tail vein contains a greater number of white blood cells than the heart blood (Table 2).

Table 2
COMPARISON OF VENTRICLE AND PERIPHERAL BLOOD IN MACDOWELL-BAGG ALBINO MICE

Sex	Total Leukocyte Count, Number per Cu. Mm.	
	Ventricle	Peripheral
♀	3,080	21,100
♀	4,100	16,980
♀	4,280	21,750
♀	3,840	17,000
♂	3,480	30,050
♀	3,220	32,460
♂	4,420	16,400
♂	3,320	16,350
Mean	3,717	21,510

BLOOD FORMING AND BLOOD DESTROYING ORGANS

Bone marrow.—Within the cavities of the bones reticular stroma forms a framework, the meshes of which are filled with marrow cells. The stroma consists of reticular cells, fixed macrophages and reticular fibers. The marrow cells give rise to the erythrocytes, the granular leukocytes and perhaps blood platelets of the circulating blood.

The erythroblasts are immature red blood cells. The youngest of these have basophilic cytoplasm and large, round, vesicular nuclei. As the hemoglobin content of these cells increases, the cytoplasm becomes polychromatophilic. They divide by mitosis and some of the cells originating from the division undergo further changes. The hemoglobin content increases still more and the cytoplasm becomes acidophilic. At the same time the vesicular nucleus becomes compact and dark staining. Such cells are called normoblasts. After losing their pyknotic nuclei they are ready to enter the circulation as erythrocytes.

The myeloblasts are large cells with large, round, vesicular nuclei, containing coarse chromatin granules, surrounded by a small amount of non-granular cytoplasm. They undergo proliferation and give rise to myelocytes which have indented bean-shapped nuclei and slightly granular cytoplasm. The myelocytes divide by mitosis and give rise to metamyelocytes or proleukocytes which have ring-shaped nuclei and cytoplasm containing somewhat coarser granules. These cells do not proliferate, but their nuclei change into irregular lobulated shapes, typical of mature polymorphonuclear leukocytes (Fig. 118). The cytoplasmic granules of the myelocytes and metamyelocytes may be eosinophilic or neutrophilic. Basophilic myelocytes are not found in mouse bone marrow (Haam, in Jaffé, 56). The neutrophilic granules are fine and stain faintly, while the eosinophilic granules are larger and stain intensely. The megakaryocyte is a giant, irregular shaped cell which has a single lobulated nucleus. It undergoes degeneration within the marrow. The theory that small processes of the cytoplasm of megakaryocytes are pinched off and enter the circulation as platelets is still under discussion. In addition to the cells described, the presence of fat cells, large mononuclear cells and lymphocytes is constant in the marrow.

Petri (77) gives the following average differential count based on the cells of the femoral marrow of 14 white mice: nucleated red blood cells 23%, myeloblasts 4.7%, myelocytes 9.2%, proleukocytes 6.5%, leukocytes 34.3%, non-identified 18.7%, large mononuclear cells 0.1%, reticulo-endothelial elements 3.3%, megakaryocytes 0.2%.

The mature myeloid cells enter the circulation by passing through the thin wall of the venous sinusoids. Arteries and veins are numerous in the bone marrow.

The femur or sternum are suitable for obtaining marrow smears for histological examination. According to Jaffé (56) the marrow of the long bones is functional throughout the life of the mouse, and is not replaced by fatty, yellow marrow.

Lymph nodes.—Lymph nodes are small, bean-shaped organs composed of lymphatic tissue and located in the course of lymph vessels. At the indented area, which forms the hilus, blood vessels enter and leave the node. Intercommunicating large lymph spaces, the lymph sinuses, are present throughout the organ. Each node is surrounded by a thin connective tissue capsule which is especially well developed at the hilus, where it may project for a distance into the medullary area. Trabeculae which are continuous with the capsule divide the cortical part into alveolar areas and the medul-

lary part into irregular spaces. In both areas reticular fibers and primitive and phagocytic reticular cells form the finer network.

In the cortex the lymphocytes may form rounded nodules which, however, are not constant structures and may vary in size and position (17), or may be entirely absent, in which case the lymphocytes are arranged diffusely without any definite structure. When a nodule is very active in producing lymphocytes, its central area is lighter staining, and among its cell components are many medium sized lymphocytes. Such areas contain mitotic figures and have been called secondary nodules or germinal centers.

In the medulla the lymphatic tissue is arranged in cords, surrounded by wide meshes of the medullary sinuses. In the cords among the lymphocytes free macrophages, eosinophils, plasma cells and occasionally mast cells are also present. The free macrophages originate from the phagocytic reticular cells (fixed macrophages) and are capable of ameboid movement. They are elongated, irregular shaped cells with oval nuclei containing coarse chromatin granules. The plasma cells have eccentric round or oval nuclei, with large, darkly staining chromatin granules distributed in a fairly regular pattern. The cytoplasm is homogeneous and slightly basophilic. The mast cells are large, oval or polyhedral cells, with small round nuclei and cytoplasm containing large granules which stain intensely with hematoxylin and often obscure the nuclei.

Arteries enter at the hilus. They usually follow the course of the trabeculae and branch repeatedly. The endothelial cells lining the capillaries are unusually tall, resembling in cross section cuboidal epithelial cells. Several afferent lymph vessels enter through the capsule at the surface of the organ. The lymph circulates throughout the sinuses, and lymphocytes produced here are carried away by this constant flow. At the hilus the lymph is collected into efferent lymph vessels.

Great variation exists in the size and shape of the lymph nodes, as well as in the relative size and position of the medulla and the cortex. Often the trabecular system is poorly developed, and the nodes contain a diffuse mass of lymphatic tissue (57).

The spleen.—The spleen is a slightly curved, finger-shaped organ covered with a capsule composed of dense connective tissue containing some smooth muscle fibers. The trabeculae project in from the capsule, and with a network of reticular cells and fibers form a framework which is filled by the splenic pulp (Fig. 40). The splenic artery divides into two branches which enter the spleen on its concave dorsal side, thus dividing the organ into three approximately equal parts. Variations exist, and one or both of the second-

ary arteries may divide forming more than two points of entrance. After entering the organ the arteries divide repeatedly, decreasing gradually in size. During their courses they give rise to numerous capillaries which supply the lymphatic tissue, the white pulp, with blood. Lymphatic tissue is arranged around the arteries in the form of a continuous sheath which thickens at points where branching occurs. Around the small arteries the tunica adventitia is replaced by lymphatic tissue. Lymphatic nodules, or

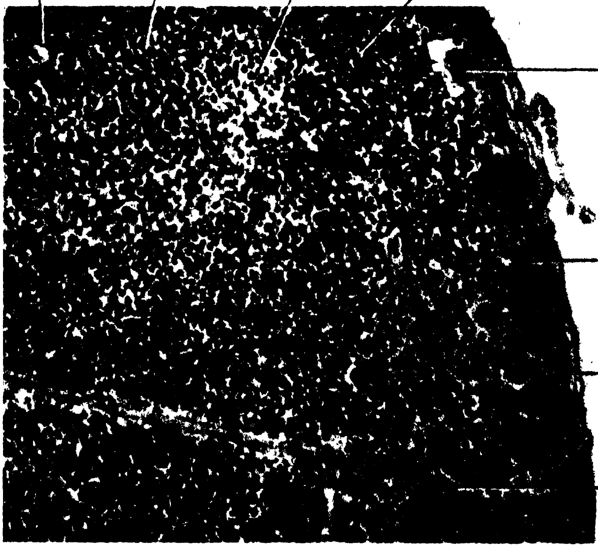


FIG. 40.—Spleen. (×50.)

splenic nodules (Malpighian bodies), are found arranged around central arteries. The outline of these nodules is usually very indistinct. Their central areas sometimes contain many medium sized lymphocytes forming germinal centers, the secondary nodules. As in lymph nodes, these are transitory structures.

The small arteries of the white pulp, after repeated branching, enter the surrounding tissue, the red pulp, where they divide into many straight arteries, the penicilli. A short distance before their termination, the walls of these vessels are thickened by closely applied fibers of reticular tissue, while the lumina remain narrow. These are the sheathed arteries of the pulp, which after further branching give rise to arterial capillaries. The question of "open" or "closed" circulation, depending on whether the arterial capillaries open into the spaces between the reticular cells or into the venous sinuses, is still under discussion.

According to Knisley (63) who observed the circulation of the living mouse spleen by transillumination, the branches of the penicilli divide into arterial capillaries. Some of these capillaries after a somewhat curved, unbranched course connect directly with venules. Others after a short course connect with the afferent ends of venous sinuses. Some of the sinuses intercommunicate, forming multiple sinus routes; others form a single sinus route. Both routes finally open into venules. The tissue between the sinuses forms the splenic cords. According to the same author, in the unstimulated spleen few erythrocytes are present in the splenic cords. These leave the closed vascular system by individual penetration of the walls. In traumatized and dying spleens rapid changes occur which result in the passage of large numbers of erythrocytes into the pulp tissue.

The red pulp fills the spaces between the terminal venous sinuses, forming the splenic cords. The framework is formed by reticular fibers, primitive reticular cells and fixed macrophages. In addition to the lymphatic elements and elements of the circulating blood, free macrophages, small groups of myelocytes, erythroblasts and plasma cells are present. Megakaryocytes are constant constituents although their number varies considerably. As the red pulp of the mouse contains many lymphocytes and few erythrocytes, it is not well delimited from the white pulp. The reticular cells of the red pulp almost always contain varying amounts of pigment.

There is great variation in the size of the spleen. The distal end of the organ occasionally shows bifurcation. Accessory splenic tissue in the pancreas or in fat lobules of the mesentery is often found.

Endocrine Glands

Hypophysis.—The hypophysis rests on a slight depression of the sphenoid bone. It is attached to the floor of the third ventricle by a short stalk (96). It consists of two main parts which are separated from each other by a narrow cleft, the residual lumen of Rathke's pouch. The part which is directly above the sphenoid bone and below the cleft is the anterior lobe or pars distalis, while the parts above the cleft are the pars intermedia and the pars nervosa (Fig. 41).

The anterior lobe is formed of epithelial cells arranged in cords or alveolar groups which are separated from each other by delicate connective tissue septa. Small cysts lined by ciliated cells have been found occasionally. The epithelial cells can be classified as chromophobe and chromophil cells. The chromophobe cells (also called chief cells) have large, light staining

nuclei, surrounded by small amounts of non-granular cytoplasm. The nucleus contains one or two intensely staining nucleoli.

There are two kinds of chromophil cells. Stained with hematoxylin and eosin, the cytoplasmic granules of some cells take the hematoxylin stain—these are the basophil cells; others take the eosin stain—these are the



FIG. 41.—Hypophysis. Fixed in 10% formalin. (×200.)

acidophil cells. The basophils are large polyhedral cells with eccentric nuclei and a large amount of cytoplasm. Variations exist in the amount and size of the basophilic granules. The cytoplasm often contains vacuoles of different size.

The acidophils are small, round or oval cells with centrally located nuclei. The nucleus contains a large acidophilic and a smaller basophilic nucleolus.

Variations exist in the relative number of the three types of cells. The number of degranulated basophil cells increases during pregnancy (61). After castration the basophil cells increase in number and in size, and in some of them cytoplasmic vacuoles are present (castration cells).

According to Severinghaus (86) there is strong evidence that the chromophobic cells are progenitors of distinct and divergent chromophilic cell types, and that no transition between the basophils and acidophils is possible. The study of castrate pituitaries indicates that the chromophils may revert to their chromophobic form.

The pars intermedia is above the cleft and is in close contact with the pars nervosa. It is very well developed in the mouse. The epithelial cells are arranged in small irregular groups. Most numerous are the polygonal cells with oval nuclei and non-granular cytoplasm. These are similar to the chromophobe cells of the pars distalis. Spindle-shaped cells often as long as the width of the pars intermedia are also present. They have dark staining oval nuclei. These cells are considered by Benda (in Jaffé, 56) as endothelial cells lining very minute capillaries. More general opinion maintains that the intermedia has poor blood supply.

The pars nervosa contains ependymal and glia cells and fibers. Elements from the pars intermedia may project into this zone. Gersh (38) describes specific parenchymatous cells which are distinguished from the neuroglia cells elsewhere in the central nervous system by their characteristic cytoplasmic inclusions. In the mouse these cells have an oval nucleus and a large prominent nucleolus. The cytoplasm contains osmophilic granules. In other cells osmophilic granules are not present, but the cytoplasm is filled instead by delicate basophilic granules which may be arranged in short chains or clumped masses. Some cells are intermediate between these two types. Gersh states that the parenchymatous glandular elements of the neuro-hypophysis produce and secrete the antidiuritic substance.

Thyroid gland.—The lateral lobes of the thyroid are situated on the sides of the trachea, just below the larynx. They are connected by a very narrow transverse lobe, the isthmus. The lobes are surrounded by a fibrous capsule. The organ is composed of follicles of varying size which are filled with colloid (Fig. 42). In section this material is homogeneous and stains well with acid dyes. The follicles are lined by simple cuboidal epithelial cells, having large, spherical, central nuclei and clear cytoplasm. Occasionally round droplets of colloid or clear vacuoles may be present in the cytoplasm. The cell outlines are distinct. It is considered that glands composed of tall cells are more active than those composed of low cells. The follicular epithelium is surrounded by the interfollicular reticular connective tissue, which is very rich in blood and lymph supply.

Parathyroid glands.—Each parathyroid gland is surrounded by and separated from the thyroid by a connective tissue capsule (Fig. 42). The

positions as well as the number of lobes is variable. Usually they are situated at the caudal end of each of the two lateral lobes of the thyroid. The gland consists of densely arranged groups or cords of polygonal cells. In some of these cells the nuclei are round and contain fine chromatin granules;

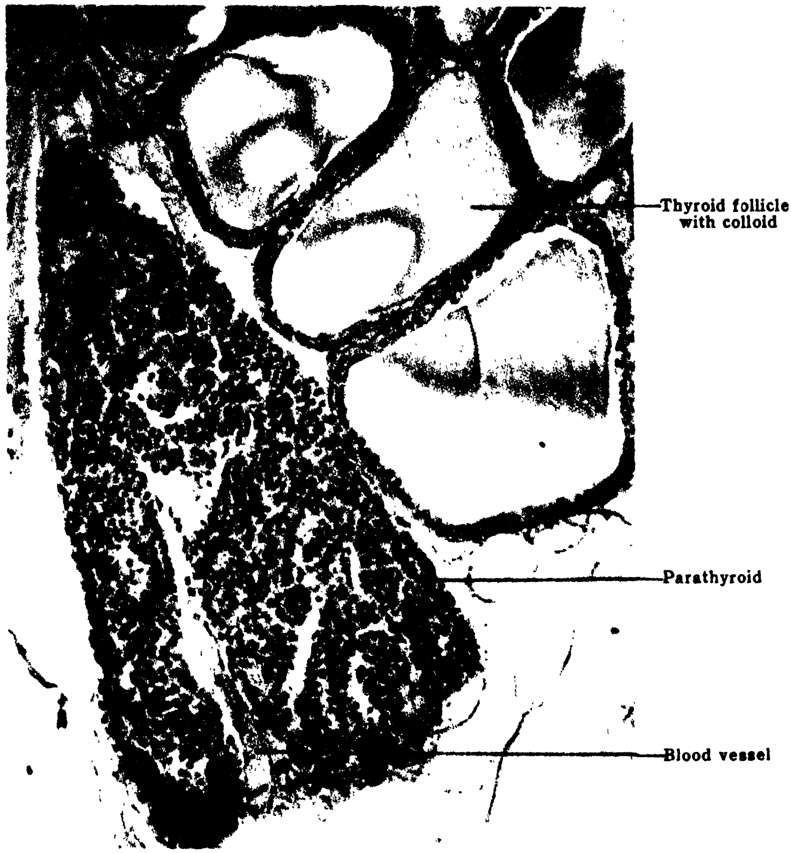


FIG. 42.—Thyroid and parathyroid glands. (×200.)

in others the nuclei are elongated and contain a single large nucleolus. According to Larionow (67), in young mice the former predominate, and in old mice the latter, while mice of middle age occupy an intermediate position. The supporting framework consists of reticular fibers and a network of capillaries.

The adrenal glands.—The adrenal glands are situated immediately anterior to the kidneys. Each gland has a thin connective tissue capsule which projects into the parenchyma and forms supporting trabeculae. A cross section shows a central medulla and a peripheral cortex. Dependent

upon the size and arrangement of the cells, the cortex may be divided into three zones. Immediately beneath the capsule is the narrow zona glomerulosa formed by small cells arranged in arch-like groups. The cells have relatively large nuclei and slightly basophilic cytoplasm. In the next zone, the wide zona fasciculata, the cells are larger and are arranged in more or less definite radial columns separated from each other by small blood



FIG. 43.—Adrenal gland. Fixed in Bouin's fluid. (×200.)

vessels. The cells have vesicular nuclei, and the cytoplasm appears foamy, due to the presence of finely distributed lipoid droplets. This zone morphologically resembles the corpus luteum. The third zone, the zona reticularis, is composed of strands of small cells which form a network (Fig. 43).

According to Howard-Miller (51), in the adult male mouse the fascicular and reticular layers are not definitely limited, and their separation into two zones is not justified. The same author observed that in the young adult nulliparous female the zona glomerulosa and fasciculata are similar to those of the male, but that in addition to these zones there exists a wide third zone composed of cells which differ from the cells of the zona fasciculata by being smaller and staining more intensely. She named this

highly developed reticular zone, which has specific variations under certain conditions, the X zone. The X zone is present in the male until about the time sexual maturity is reached. It persists in castrated males at least four months longer. In the female it continues to develop until at 4–5 weeks it occupies a much larger area than in the male of the same age. The zone disappears during first pregnancy, but in virgin females it persists for a longer period, gradually degenerating between the third and seventh month. The gradual degeneration leads to hyperemia and widespread vacuolization and the separation of the medulla and cortex by connective tissue. In a later article (52) the same author states that: "Mice of different strains show considerable variation in the amount of adrenal X zone tissue which they normally develop."

The cells of the medulla are arranged in rounded groups and wide reticular cords, separated from each other by sinusoidal blood spaces. The cells and consequently the reticular cords are considerably larger than those in the zona reticularis of the cortex. The nuclei are large and centrally located and the cytoplasm is pale staining. If the gland is treated with potassium dichromate, small brown granules are visible in the cells. Chromic acid stains the cells evenly brown, giving the so-called chromaffin reaction.

Arteries enter the gland from the capsule. They form the capillaries of the cortex. The sinusoidal blood spaces of the medulla drain into the tributaries of a large central vein and leave the gland at the hilus.

The presence of small accessory adrenals in the vicinity of the gland is not infrequent.

Thymus.—The thymus is situated in the thorax ventral to the aortic arch. It consists of two lobes of unequal size lying close together. The lobes are covered by fibrous connective tissue from which septa project in and produce lobulation without dividing the gland into distinctly separate lobules. In a cross section of the thymus a light staining medulla and a dark staining cortex can be differentiated (Fig. 44). In both parts reticular cells form a supporting framework. In the cortex densely packed, small, round cells are present. These cells are considered identical morphologically with small lymphocytes by some authors, while others consider them of epithelial origin and call them small thymocytes. They have dark staining, slightly eccentric, round nuclei with dark chromatin granules and prominent nucleoli, surrounded by a very small amount of cytoplasm. Because of the dense arrangement of these cells, the reticular cells are difficult to see.

Toward the medulla the density of the thymocytes changes rather suddenly and the light staining reticular cells become much more prominent. These cells are entodermal in origin and their epithelial character is evident during embryonic life. The reticular cells have pale spherical nuclei with fine chromatin granules and indefinite cell outlines. Thymocytes are present, although not densely arranged. Eosinophils and plasma cells are usually found here. Small groups of large, pale staining epithelioid



FIG. 44.—Thymus. (×200.)

cells with large oval nuclei occur in the medulla. These, however, are not flattened and concentrically arranged like the typical Hassall's corpuscles. Cysts of varying sizes are frequently present. The smaller cysts are often lined entirely by cuboidal, ciliated cells; in the larger ones the lining consists partially of flat, partially of ciliated cuboidal cells.

The gland is relatively large during embryonic life and in postnatal life up to the time of puberty but undergoes involution after sexual maturity is reached. During involution the thymic cells of the cortex gradually become less dense and the division into cortical and medullary part is less prominent. Adipose tissue is not deposited in the involuted thymus of the mouse.

Arteries enter the capsule, are distributed first to the cortex, then to the medulla. Veins arise in the medulla and leave the organ at the hilus.

Pineal body.—The pineal body (epiphysis cerebri) is a small, cone-shaped body which is situated above the roof of the third ventricle and is attached to its posterior part by a stalk. The gland has a fibrous connective tissue

capsule, from which septa project into the parenchyma and divide it into irregular areas. The cells are arranged in cords and spherical groups (Fig. 45).

Neuroglia cells with long stellate processes form a reticular framework for the cell cords. Some of the cells of the cords have compact, small, round, dark staining nuclei and homogeneous cytoplasm. Others have indistinct cell outline and possess large, pale staining, oval nuclei with finely

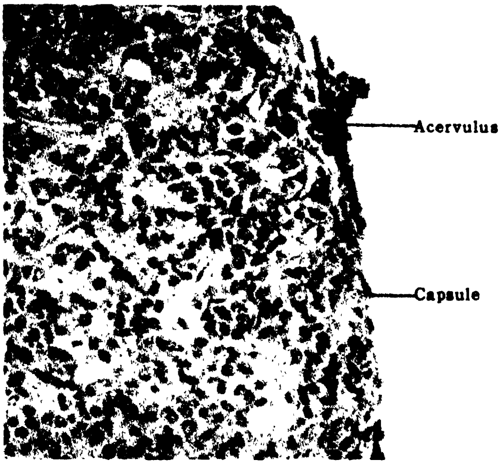


FIG. 45.—Pineal body. (X200.)

distributed chromatin granules. Sand granules (acervuli) are present in old animals, situated beneath the capsule or in the center of cell groups. The blood supply is rich and small capillaries form a dense network around the glandular cells.

SKIN AND HAIR

The skin.—The skin is composed of two parts, the epidermis which is a stratified squamous epithelium, and the corium or dermis, the underlying connective tissue. The epidermis of the mouse is very thin at all surfaces where hair covers and protects the animal. At areas where the hair is thin or absent (as at the anus, around the nipples, tail, feet, etc.) the epidermis is considerably thicker.

Around the nipple the epidermis consists of about ten to twelve layers of epithelial cells. The cells of the basal layer which are next to the corium are columnar in shape and are placed perpendicularly to the skin surface. They have large oval nuclei and frequently show mitotic figures. The next few layers of cells are polyhedral or flattened squamous cells. These cells

and the basal cells together form the stratum germinativum (also called stratum Malpighii or stratum spinosum). The cells of this layer are separated from each other by intercellular spaces. Spines protruding from the cell surface form bridges connecting the cells with each other, and the spines of the basal cells penetrate the connective tissue of the dermis.

The next three to four layers of cells in the epidermis of the nipple are flattened, have light staining oval nuclei, and possess cytoplasm containing coarse keratohyalin granules. These cells form the stratum granulosum. The intercellular spaces between these cells are considerably narrower, gradually becoming indistinct.

The next layer consists of about four to five layers of flattened, dead, cornified cells, the peripheral layers of which are constantly in the process of desquamation. This layer is the stratum corneum.

The surface of the dermis forms projecting elevations, the dermal papillae, which project into the epidermis. The dermis is composed of fibrous connective tissue and adipose cells. It is rich in blood supply.

Where the epidermis is thin some of the strata described above are not present (Fig. 81). The cells of the stratum germinativum are cuboidal or polyhedral in shape and form only about one to two cell layers. The stratum granulosum is absent. The stratum corneum consists of one to two layers of cornified cells. Dermal papillae are absent.

Below the dermis loose connective tissue and adipose cells form the subcutaneous layer. Where the panniculus carnosus is present, the striated muscle fibers of this layer separate the cutaneous layer from the subcutaneous connective tissue. At other places the subcutaneous tissue is a loose continuation of the dermis.

Pigment is present in the basal cells of the epidermis and in the cells of the dermis of the tail and ear in many different strains.

The hair and the vibrissa.—The part of the hair which projects above the skin surface is the hair shaft; the part within the skin is the root. The root is enclosed in a tubular sac, the hair follicle, which is composed of both the epidermal and the dermal layers of the skin. The dermal part of the follicle is continuous with the papilla, which projects into the basal part of the hair root. The epithelial cells around the papilla form the hair matrix. These cells multiply, move upward and are responsible for the growth of the hair. The cytoplasm of these cells, in animals which have colored fur, contains pigment.

As the histological details are essentially similar to those in human hair, for a description of the cell layers of the hair the reader is referred to

Maximow and Bloom (73). Dry (30) describes the development and succession of the various types of overhairs, underfurs (zigzags), vibrissae, hairlets, and hairs of the arioles of the mouse.

Vibrissae, tactile hairs or sinus hairs are long coarse hairs with deeply imbedded hair follicles (22, 23). Between the connective tissue sheath and

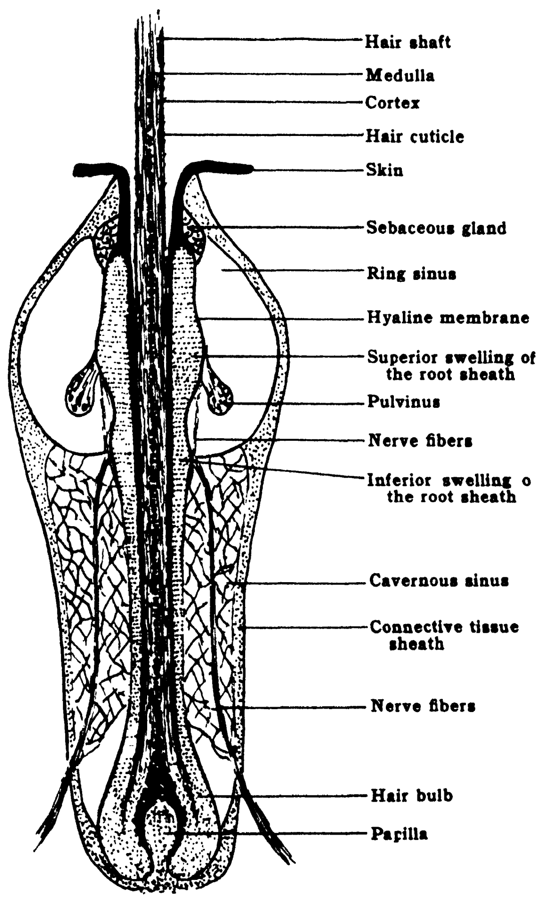


FIG. 46.—Diagram of a vibrissa. (×125.)

the hyaline membrane of the hair follicle a cavity is present, the lower part of which is divided into reticular spaces by a network of fibers originating from the inner surface of the fibrous sheath, while the upper part contains one circular space (Fig. 46). These cavities are filled with blood and form the cavernous and circular (or ring) sinuses respectively. The cells forming the reticular network often contain pigment. Extending into the circular sinus is an oval-shaped projection, the pulvinus. According to

Vincent (94) this is much shrunken in prepared sections, but in expanded state surrounds the follicle entirely and fills in the space between the walls. The root sheath shows two slight enlargements forming the superior and inferior swellings. Vincent stated that many nerve fibers have their terminations here.

Striated muscle fibers surround the connective tissue sheath. The contraction of the muscles around the opening of the follicle permits the hair to vibrate freely. Muscle fibers connect the walls of adjacent follicles and are responsible for the uniform, almost continuous movement of these hairs.

Hibernating, Lacrimal and Harderian Glands

Hibernating glands.—Some of the adipose tissue differs from the usual white fat and has a characteristic light brown color which is due to the presence of pigment. It is divided into lobules by loose fibrous connective tissue and was considered to be a gland of internal secretion by the early investigators. Many different names were suggested for it, among them hibernating gland, interscapular gland, multilocular adipose tissue, oil gland, etc. It is present as a large bilobed mass between the scapulae, in the superior mediastinum about the thymus, in the cervical region, in each axillary fossa, and in the abdominal cavity as perirenal lobes (80).

The fat cells have large, round, centrally located nuclei with fine chromatin granules. The distribution of fat is multilocular, being present in the form of numerous small droplets. The abundant granular cytoplasm contains small spherical vacuoles (dissolved fat droplets), the outline of which stains intensely with eosin. Groups of cells are supported by reticular fibers which also surround the capillaries. The blood supply is rich.

According to Rasmussen (80), "The structural differences such as the more granular character of the cytoplasm of the cells . . . are not sufficient evidence to warrant the conclusion that the structure under consideration is of any endocrine significance."

Lacrimal glands.—The exorbital lacrimal glands are situated slightly below and in front of the ears. The gland is tubulo-alveolar and is composed of small lobes, which are enveloped and divided into lobules by fibrous connective tissue (Fig. 47). Structurally the gland resembles the parotid gland. It differs from it in that its intra-lobular ducts are lined by low cuboidal epithelial cells, which lack basal striations. The alveoli are slightly larger and more loosely arranged than the similar structures of the parotid gland.

The main duct leads toward the posterior corner of the eye. Here the duct joins the intra-orbital lacrimal gland which consists of a small lobe, and is identical structurally with the exorbital gland.

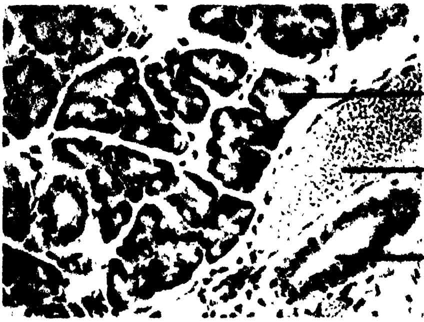


FIG. 47.—Exorbital lacrimal gland. (×200.)

Harderian glands.—The Harderian gland lies behind the eyeball and partially encircles the optic nerve. It is tubulo-alveolar in structure (Fig. 48). A thin connective tissue membrane surrounds and divides the gland into lobes and lobules. The tubules and alveoli are lined by tall



FIG. 48.—Harderian gland. (×200.)

columnar epithelial cells in which the pale staining round nuclei are at the bases of the cells. The cytoplasm contains minute fat droplets which are seen in sections as small vacuoles, separated from each other by acidophilic granules. In many tubules the cells are broken down and the lumina contain a fatty secretion which is the product of cell degeneration.

The gland cells rest on a delicate lamina propria, the cells of which occasionally contain pigment granules. The pigment may color the secretion present in the lumina and is visible grossly as small dark granules and

in section as homogeneous brown drops. The short excretory ducts are lined by cuboidal epithelial cells and open at the base of the nictitating membrane.

Oral Cavity and Associated Structures

The oral cavity.—The lips are covered on the outside by skin containing deeply imbedded hair follicles. At the zone of transition from the skin to the mucous membrane covering the inner surface of the lips, the hairs disappear and the stratified squamous epithelium becomes much thicker, its outer layers being cornified. Similar epithelium lines the entire oral cavity. Below the epithelium the fibrous lamina propria forms low, broad papillae. At the corners of the mouth there are large sebaceous glands which open directly through short ducts to the surface of the lips.

The dental formula of the mouse is: incisor 1/1, cuspid 0/0, premolar 0/0, molar 3/3. The incisors in both jaws are bow-shaped with the root projecting far back below the root of the third molars. According to Weber (in Jaffé, 56) the crown on the outer convex side is covered by enamel, while on the inner concave side the enamel is absent and the dentine is covered by cementum. As the incisors are growing continuously, their apical foramina stay open. The molars are similar structurally to the human molars.

The anterior part of the roof of the mouth, the hard palate, bears rows of membranous ridges. The three anterior ridges are transverse; the five pairs of posterior ridges are V-shaped. They are covered by stratified squamous epithelium showing keratinization, and are supported by the dense fibrous lamina propria which takes part in their formation. The mucous membrane is firmly attached to the surface of the bones.

The posterior part of the roof of the mouth, the soft palate, is composed of striated muscle fibers and fibrous connective tissue covered by mucous membrane. On the oral surface and at the posterior margin of the soft palate the epithelium is cornified stratified squamous, while toward the nasal surface, a short distance from the margin, this changes into columnar pseudostratified, and still farther into pseudostratified ciliated columnar respiratory epithelium. Mucous glands are present on the oral surface beneath the mucous membrane. They are surrounded by loose vascular connective tissue and open through short ducts into the oral cavity.

The tongue.—About one third of the distal part of the tongue lies free in the oral cavity. Farther caudad it is attached to the floor of the mouth and the wide proximal part is attached also on the sides, here forming the floor of the mouth cavity. The tongue is covered by stratified squamous

epithelium, the superficial layers of which are cornified. Except for a small proximal part, the dorsal surface is covered by small elevations, the papillae (Fig. 49). Morphologically three kinds of papillae can be distinguished. The filiform papillae are the most numerous, the fungiform papillae being



FIG. 49.—Tongue with papillae and taste bud. (×400.)

present in much smaller numbers. A single circumvallate papilla is situated on the midline close to the base of the tongue. The cone-shaped filiform papillae are formed entirely of epithelial cells. The parts projecting above the surface are composed of overlapping cornified cells. The fungiform papillae are elevated only slightly above the surface (Fig. 49). The epithelium as well as the underlying lamina propria takes part in their formation. The free surface is covered by three to four rows of epithelial cells. At its center, each papilla has a single taste bud (75). The circumvallate papilla is surrounded by a deep circular groove and does not project above

the surface. The stratified squamous epithelium lining both walls of the circular groove lacks the superficial cornified cell layers and contains numerous taste buds. These are barrel-shaped structures occupying the thickness of the stratified epithelium. Two kinds of cells take part in their formation: the tall peripheral supporting cells which have pale staining oval nuclei, and the slender neuroepithelial cells which have dark staining, spindle-shaped nuclei and end in hair-like processes. The former cells enclose a small central opening, the taste pore, into which the hair-like processes of the neuroepithelial cells project. The taste buds of the fungiform papillae are similar in structure, but differ in that they project below the epithelium into the lamina propria (Fig. 49).

Loose connective tissue forms the lamina propria, which is thin except where it projects into and takes part in the formation of the fungiform and vallate papillae. Below the propria is the musculature of the tongue. This consists of vertical, longitudinal and transverse striated muscle bundles. Blood vessels branch between the muscle layers, and in the lamina propria capillaries are numerous. Only near the base of the tongue are glands present, surrounded by and separating the muscle bundles. Those near the vallate papilla are serous glands (the glands of Ebner) which have short ducts opening at the base of the groove of the papilla. There are small lobules of mucous glands farther laterally and also dorsally which open with short ducts directly on the surface of the tongue.

The pharynx.—The oral cavity opens caudally into the pharynx, which connects it with the esophagus. The pharynx also serves as a connection between the oral and nasal passages and the larynx. Except for a small area, where the respiratory epithelium of the posterior nares persist, the entire surface of the pharynx is lined by stratified squamous epithelium with cornified superficial layers. The lamina propria is composed of dense connective tissue and is directly continuous with the muscular wall, which is composed of striated muscle fibers. Between the muscle fibers groups of mucous glands are present which open to the surface through short ducts, lined by stratified squamous epithelium. Lymphatic tissue is not present (75).

Submaxillary glands.—The submaxillary glands are two large lobes which slightly overlap on the midventral line of the neck. They are compound, branched tubulo-alveolar glands. Each lobe is divided into several lobules which are surrounded by and separated from each other by connective tissue membranes. The glands have an extensive duct system. The main duct of each lobe opens on the floor of the mouth. At its orifice

the duct is lined by stratified squamous epithelium which toward the gland changes into pseudostratified columnar type. The interlobular ducts are lined by columnar epithelial cells. The intralobular ducts are the so-called striated tubules, and are lined by rodded epithelial cells. These cells have centrally located, large, round nuclei and characteristic basal striations in their cytoplasm. The central intralobular ducts divide into terminal tubules which in turn connect with the alveoli. The alveoli are composed of "special serous cells" (18). They are pyramidal in shape and have large, oval, darkly staining nuclei near the bases of the cells. The granular cytoplasm is basophilic and unlike the serous cells of the parotid gland it does not contain chromophil substance (18). The cells rest on a basement membrane. Scattered between this membrane and the epithelial cells, stellate basal cells or "basket" cells are present.

According to Oppel (Vol. III, 57) the submaxillary glands of the rat and mouse are serous glands and do not contain any mucous cells. He states that even those cells which resemble them are not true mucous cells. Stormont (in Cowdry, 18) considers all the cells of the submaxillary gland of rabbit, rat, mouse and muskrat as "special serous cells" and gives the definition of this type of cells as "those non-mucous cells which differ in important respects from the serozymogenic type but which, notwithstanding a vast amount of research, remain, as yet, functionally and cytologically ill defined." According to the same author the special serous cells forming the gland of the mouse are of two types: "The gland tubules are composed of tropochrome cells, but the terminal segments of the intralobular ducts contain in their cells large coarse, highly refractive granules similar to those present in the homeochrome cells of the rabbit's submaxillary gland." He describes the tropochrome cells of the rabbit's submaxillary as clear, palely stained cells which show coarse reticular structure. The nucleus is usually shrunken and basal in position in fixed material. In preparations fixed in sublimate these cells present an appearance very similar to mucous cells, from which they differ by the fact that the contents of the cell spaces do not stain with any of the ordinary staining reagents for mucin. The homeochrome cells of the rabbit, according to him, have large oval nuclei situated at the bases. Unlike the serozymogenic cells, they do not contain chromophil material. The cytoplasm is abundant and filled with darkly staining large granules.

Our own observations which include the examination of the submaxillary glands of more than 200 animals from the dba and C57 black strains showed differences in the structure of the adult normal male and female animals.

The central intralobular ducts of both sexes are lined by rodded epithelium. In the adult female this type of epithelium also lines the terminal tubules into which the central intralobular ducts divide (Fig. 50A). In the adult male the lining of the terminal tubules and some of the alveoli opening into them consists of tall columnar epithelial cells with the nuclei near and often flattened against the bases (Fig. 50B). These cells resemble mucous cells

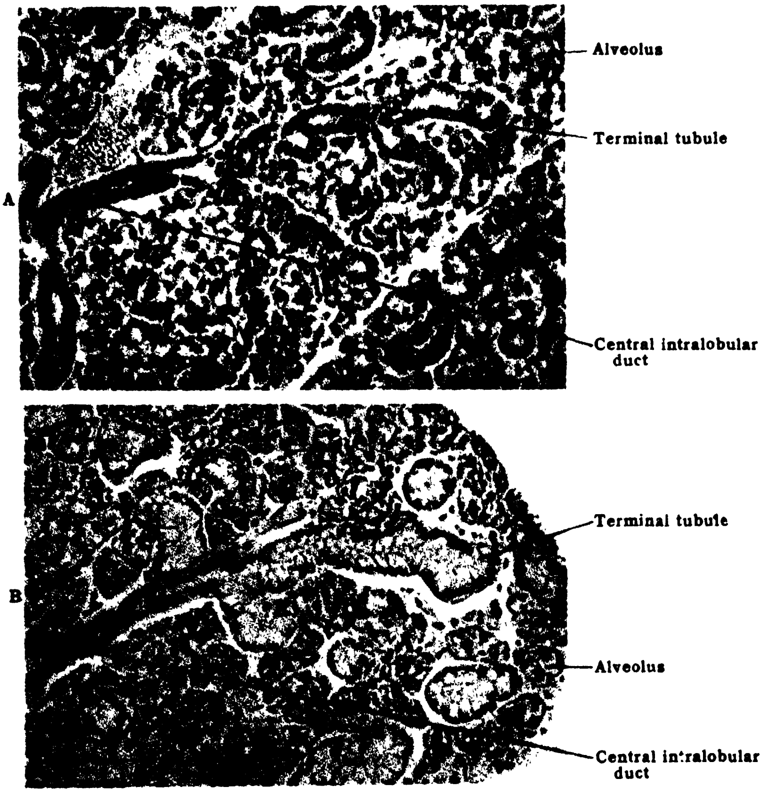


FIG. 50.—Submaxillary gland. A. Female mouse. B. Male mouse. (×200.)

and are perhaps identical with the tropochrome cells described by Stormont. They do not stain red with Mayer's mucicarmine stain. In young animals of both sexes up to about five to seven weeks the structural differences do not exist, and the tall, light staining cells are not present.*

* The above is based on observations by the author and Paul Ossen. Since these observations were recorded a recent paper by Lacassagne (65) which describes the same dimorphic structural sex differences has come to our attention.

Blood vessels ramify in the interlobar connective tissue. They follow the course of the ducts, and a capillary network provides the tubules and alveoli with a rich blood supply.

Major sublingual glands.—The major sublingual glands are in close proximity to the lateral surface of the submaxillary glands.

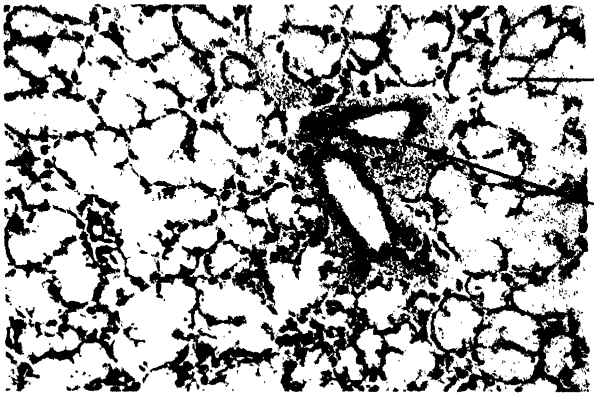


FIG. 51.—Sublingual gland. (×200.)

Loewenthal (69) referred to this gland as the retrolingual gland and considered it to be an accessory submaxillary gland. It is composed usually of one large lobe divided into smaller lobules by connective tissue septa. The main excretory duct is lined by pseudostratified columnar epithelium and has a parallel course with the duct of the submaxillary gland. It opens through a separate orifice in close proximity to the submaxillary duct. The intralobular ducts are striated tubules and are lined by rodded epithelium. The short and narrow intercalated ducts are lined by very low cuboidal epithelial cells. In the mucous cells which constitute the alveoli the nuclei are flattened to the bases and the cytoplasm appears clear and slightly basophilic. Stained with thionin the cells contain a purple-red, with Mayer's mucicarmine a red network of precipitated mucigen. The delicate basement membrane and stellate basal cells are like those described in the submaxillary gland (Fig. 51).

Parotid glands.—The paired parotid glands are composed of several small elongated lobules. Extending from the ventro-lateral surface of the neck, the posterior lobes reach the shoulders. The main duct is formed by several branches and opens in the oral cavity opposite the molar teeth (43). The intralobular ducts are striated tubules, lined by rodded epithelial cells. The intercalated ducts are lined by low cuboidal epithelial cells. The serous cells of the secretory alveoli are pyramidal in shape. Around and below

the nucleus the cytoplasm contains chromophil substance, staining intensely with basic stains and causing vertical striations. The nucleus is relatively large and round or oval in shape. Above the nucleus coarse zymogen granules are present and can be demonstrated by special technique. Basal cells are present between the epithelial cells and the basement membrane (Fig. 52).

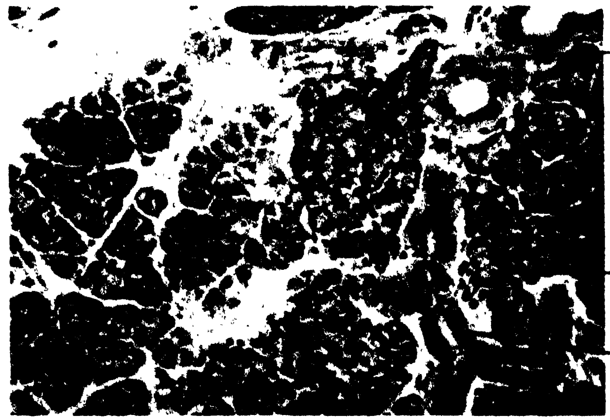


FIG. 52.—Parotid gland. (×200.)

DIGESTIVE TUBE

The wall of the digestive tube is composed of several layers. The innermost layer is the mucous membrane or tunica mucosa, which consists of a surface layer of epithelium and the underlying connective tissue, the lamina propria (also called stratum proprium or tunica propria). In some parts of the tube a layer of smooth muscle fibers, the muscularis mucosae, forms the limit of the mucous membrane and separates it from the submucosa, which is composed of loose connective tissue. Where the muscularis mucosae is not present the lamina propria changes gradually into the submucosa. The muscularis externa, also called tunica muscularis, consists of layers of muscle fibers. In the stomach and intestines this layer is surrounded by the serosa, composed of a thin connective tissue membrane and covered by mesothelium. The esophagus and the rectum are attached to the adjacent tissue by a layer of loose connective tissue, the tunica adventitia.

Esophagus.—The esophagus is a tube which connects the pharynx with the stomach. The stratified squamous epithelium lining the lumen consists of a thin stratum germinativum, a somewhat heavier stratum granulosum and a stratum corneum which forms about one half of the total

thickness. The lamina propria is composed of fibrous connective tissue and does not form papillae. The mucous membrane forms longitudinal folds. The muscularis mucosae is developed only in the lower, caudal third while in the upper two thirds the lamina propria is continuous with the loose connective tissue of the submucosa. The muscularis externa is composed of striated muscle fibers throughout the entire length of the tube to the

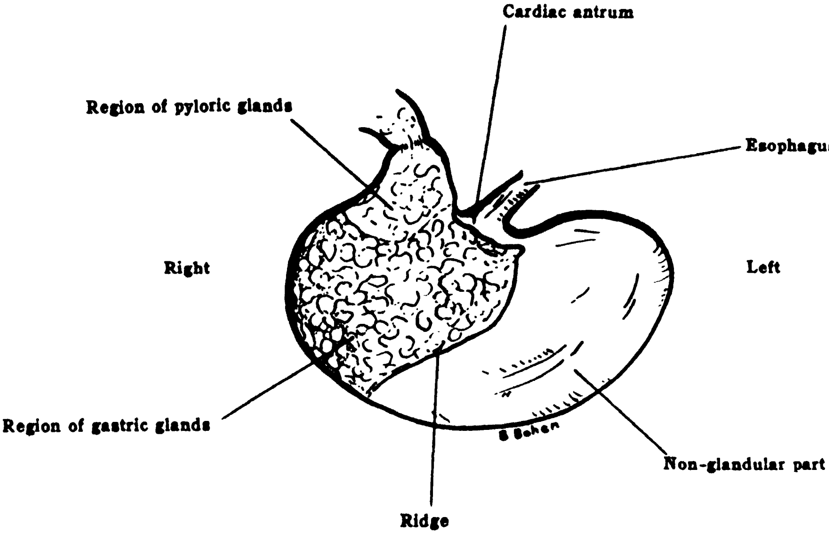


FIG. 53.—Stomach, outline drawing.

cardiac opening of the stomach. The outer surface of the esophagus is attached by a layer of loose connective tissue, the tunica adventitia. No glands are present (39).

Stomach.—The esophagus enters the stomach in about the middle of the lesser curvature. Grossly the stomach shows two parts, the thin-walled, slightly transparent, grayish part on the left, and the thick-walled, white part on the right. The mucous membrane of the former is devoid of glands, while the latter contains the digestive glands (Fig. 53). Since the wall of the stomach distends and stretches easily, the size relationship of the two parts is not always the same but depends on the amount of food present in each. The lining of the glandless part is a stratified cornified squamous epithelium similar to the lining of the esophagus. The lamina propria forms numerous papillae. At the boundary of the non-glandular and glandular part the mucous membrane of the former forms a ridge. This ridge is particularly prominent dextral to the entrance of the esophagus, there forming a channel-like extension of the non-glandular part, the cardiac

antrum. It probably has the function of directing the swallowed food toward the non-glandular part for storage.

The glands of the stomach are compactly arranged, simple, branched, tubular glands lying parallel to one another, perpendicular to the surface and occupying the thickness of the mucous membrane. On the surface of the stomach a multitude of small depressions form the gastric pits, foveolae



FIG. 54.—Stomach, region of ridge between non-glandular and glandular part. (× 100.)

gastricae. Into the bottom of each pit small groups of gland tubules open through slight constrictions.

The inner surface of the glandular stomach is covered, and its pits are lined, by tall simple columnar epithelial cells containing mucigen. They have large oval nuclei, located in the lower halves of the cells. In sections the cytoplasm above the nucleus shows faint granulation.

The glandular area of the stomach may be divided into two main parts, the larger containing the gastric glands, also called fundic glands, and the smaller containing the pyloric glands. At the junction of the glandless and glandular areas the stratified squamous epithelium covering the ridge is replaced by simple tall columnar cells (Fig. 54). Here a very short transitional zone exists where two to three rows of gland tubules are present,

lined by simple columnar cells which do not show special secretory granules. Bensley (7, 8) considered these as cardiac glands.

The gastric glands have a fairly straight course and open into short gastric pits. The cells which are most numerous in the lower third of the tubules are the serous chief cells or zymogenic cells. They are columnar cells having large oval nuclei, situated at the center, and granular cytoplasm.

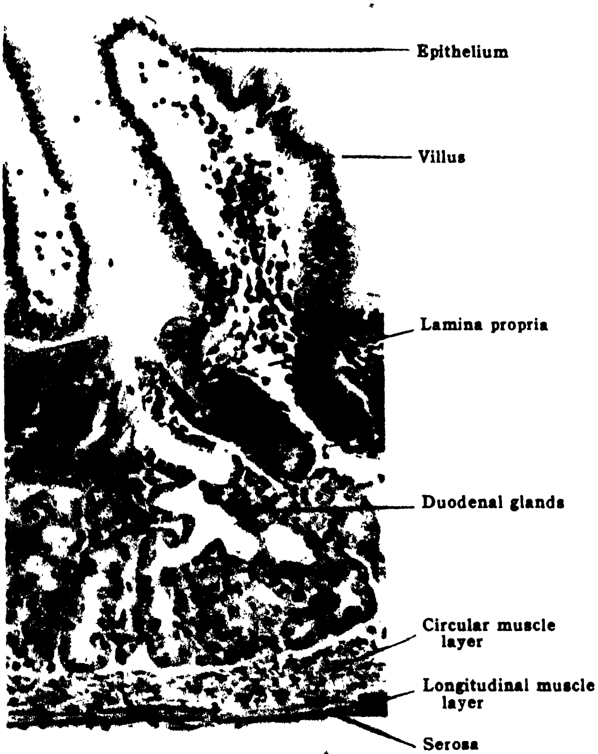


FIG. 55.—Duodenum, longitudinal section. (×200.)

In stained preparations the cells show basophilic granules above and basal striated chromophil substance below the nuclei. Mitotic division cannot be found among these cells. In the upper part of the tubules and in the neck there are cells which are similar in structure but contain lighter staining cytoplasmic granules and slightly compressed nuclei. These are the mucous neck cells. Mitoses occur occasionally among these cells. Scattered among the serous chief and mucous neck cells but occurring in particularly large numbers in the upper part of the tubules are the parietal cells which are large, round or wedge-shaped cells with clear acidophilic cytoplasm. The

nuclei are large and round and may be found two in a cell. Mitoses are not found among them.

The gastric pits of the pyloric region are deep and the gland tubules are short. The glands are lined by columnar cells in which the nuclei are near the base and the cytoplasm contains fine granulations. These cells resemble the mucous neck cells of the gastric glands and are mucous cells. The transition between the fundic and pyloric glands is gradual.

The lamina propria of the glandular part of the stomach separates the gland tubules from each other, forms the walls of the foveolae and fills the spaces between the glands and the muscularis mucosae. It consists of connective tissue containing fibroblasts, lymphocytes, some eosinophil leukocytes and plasma cells. The muscularis mucosae in both the glandular and non-glandular parts consists mainly of longitudinally arranged, smooth muscle fibers. In the glandular part thin strands of smooth muscle project between the glands. The submucosa is composed of loose connective tissue and contains blood and lymph vessels. The muscularis externa is thin in the non-glandular and better developed in the glandular part. It consists of an irregular inner oblique, a well developed middle circular and a thin outer, longitudinal, smooth muscle layer. The circular layer is particularly well developed at the pylorus. About the organ is a serous membrane consisting of loose connective tissue containing adipose cells and covered by mesothelium. Frequently a solitary lymph node is present in the serosa at the lesser curvature.

The small intestine.—The small intestine extends from the pyloric valve to the caecum (about 18 inches). It may be divided into three parts: the duodenum, the jejunum and the ileum.

The inner surface of the small intestine is covered with villi, delicate finger-like projections of the mucous membrane. Plicae circulares are not present. The villi of the duodenum are tall and leaf-shaped, being wide at the base and narrow at the tip (Fig. 55). In the jejunum and ileum they are cylindric in shape, tall in the former and short in the latter (Fig. 56). Between the villi are the openings of the simple tubular intestinal glands (crypts of Lieberkühn). The surface of the villi and the areas between them are covered by simple, very tall, columnar epithelial cells, having oval nuclei situated in their lower thirds and striated cuticular borders at their free surfaces. These epithelial cells continue into the glands, becoming somewhat shorter near and at the base. The cells near the base of the glands show numerous mitoses. Oval-shaped goblet cells are present, scattered among the columnar cells. The nuclei are pushed to the base,

and the mucigen-containing upper parts of the cells are distended. They are especially numerous in the ileum. Some of the cells lining the bases of the tubules contain acidophilic cytoplasmic granules above their nuclei. These are the cells of Paneth, which become more conspicuous after several hours of fasting and are most frequent in the jejunum.

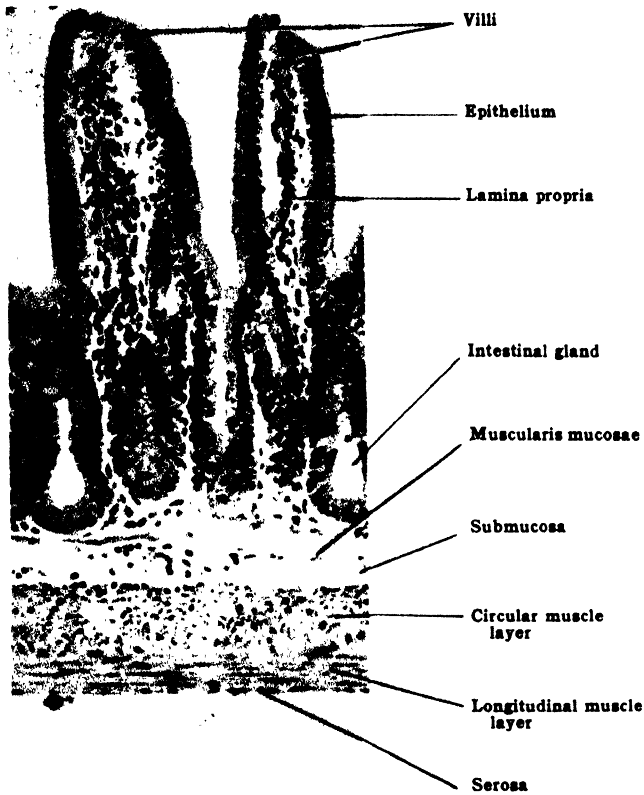


FIG. 56.—Ileum, longitudinal section. (×200.)

The lamina propria forms the center of each villus and fills the spaces between the glands. It is composed of reticular tissue containing many lymphocytes, some granular leukocytes (especially eosinophils), and plasma cells.

The muscularis mucosae is very delicate. The submucosa consists of loose connective tissue. In the submucosa of the pyloric valve and extending for a few millimeters along the wall of the duodenum, coiled, tubulo-alveolar mucous glands are present. These are the duodenal glands (of Brunner) (Fig. 55). Although they are located in the submucosa, some parts of the glands may be present in the mucosa. The glands are lined

by cuboidal epithelium with spherical nuclei and pale staining cytoplasm. Their excretory ducts open at the bases of the intestinal glands. In the submucosa near the entrance of the pancreatic duct small groups of pancreatic acini are often present. An inner well-developed circular and a thin outer longitudinal smooth muscle coat covered by the serosa complete the wall.

Solitary lymph nodules occur in the lamina propria of the small intestine. Aggregations of lymph nodules known as Peyer's patches also occur. Where these are present the villi are absent or shortened. These aggregated nodules extend into the submucosa and are covered only by the thin muscle coat and serosa. They cause a bulging of the outer surface which is visible grossly. Our observations are in accord with those of Hummel (54) in rats, who found that variations exist in the number and location of the patches, although in position the nodules are usually opposite the attachment of the mesentery.

Each villus usually contains a central, endothelial-lined, lymphatic vessel, the lacteal, which drains the absorbed fat or the white lymph (chyle). The lacteals anastomose with lymph vessels of the muscularis mucosae.

The large intestine.—The large intestine consists of the caecum, the colon and the rectum.

The caecum.—The caecum is a curved, blindly ending sac which communicates with the ileum and the colon. At its inner curvature it has several transverse folds, while most of its surface is smooth. Villi are not present. The epithelial cells of the lumen and the glands are like those in other parts of the large intestine. The lamina propria of the proximal part contains few lymphocytes. The muscularis mucosae is well developed and takes part in the formation of the transverse folds. The distal, blind end contains an aggregation of lymphatic tissue between the surface epithelium and muscularis externa. The inner circular, smooth muscle layer of the muscularis externa is well developed, while the outer longitudinal layer is thin. The serosa is like that of the small intestine. The caecum does not end in a typical vermiform process.

The colon and the rectum.—The colon and the rectum are devoid of villi. Except that the tubules are straighter and slightly longer, the glands of the large intestine are similar in structure to those of the small intestine. Goblet cells are present in large numbers, while the cells of Paneth are absent. The free surface between glands is covered by simple columnar epithelial cells with striated borders. The lamina propria contains the same elements described in the small intestine. The muscularis mucosae is

poorly developed in the colon and well developed in the rectum. The muscularis externa and the serous membrane are like those of the small intestine. Taeniae are absent (56).



FIG. 57.—Colon, longitudinal section. (×200.)

Solitary lymph follicles are present in varying numbers. They extend into the submucous layer. Peyer's patches are also found. The ascending part of the colon contains several (about fourteen) parallel transverse folds, which are made up of the mucous membrane (Fig. 57). In the descending colon and rectum the fecal material is pellet-shaped. Where pellets are found the lumen is slightly distended and smooth, while between them longitudinal folds (colic ridges) are present (55). The first part of the

rectum is similar structurally to the colon. There is a gradual increase in the thickness of the inner circular smooth muscle layer of the muscularis externa. The serous covering is replaced by the loose connective tissue of the adventitia, which attaches the rectum to the surrounding tissue. Toward the anal opening the glands become shorter and disappear as the lining of the lumen changes into thick, stratified squamous epithelium which is continuous with the thin, stratified squamous epithelium of the skin. The musculature at the anus is composed of striated muscle fibers. Surrounding the anus are the anal glands. They are similar structurally to sebaceous glands and are arranged in small lobules separated by connective tissue.

Mesenteries.—The stomach and the intestines are attached to the abdominal wall by the mesogastrium (omentum) and mesenteries respectively. These are thin, transparent membranes composed of loose connective tissue containing many adipose cells, lymphocytes and granular leukocytes, and covered on their free surfaces by mesothelium. They contain many blood and lymph vessels.

Arteries enter and leave the intestinal walls through the mesentery. In the submucosa they form a network which in the small intestine gives off two kinds of branches, both of which enter the muscularis mucosae. Some of the arterial branches supply the intestinal glands with dense capillary networks, others supply the villi. The latter enter the base of each villus and form a capillary network which is in close proximity to the epithelium. At the tip of the villus the capillaries collect into veins which have a parallel course with the arteries.

Liver and Pancreas

The liver.—The liver consists of four main lobes: a large median, a right and left lateral and a left caudal. All the lobes except the left lateral lobe are partially divided by deep bifurcations. The gall bladder is attached to the caudal surface of the median lobe.

From the very thin connective tissue capsule covering the lobes, strands of connective tissue project into the gland and form the supporting framework, or capsule of Glisson, for the interlobular vessels and bile ducts. This framework is very poorly developed and divides the gland into indistinct polygonal areas, the lobules. In the center of each lobule is the central vein, surrounded by anastomosing, radially arranged cords of liver cells (Fig. 58). The central veins are intralobular tributaries of the hepatic veins. The interlobular vessels which are surrounded by the capsules of

Glisson are branches of the hepatic artery and of the portal vein. According to Higgins (47), "Two rather large hepatic ducts accompany each main branch of the portal vein, through the lobes of the liver, while usually but a single one follows the smaller distal branches of the vein. Branches of the hepatic vein are not associated with bile ducts."

The cords of liver cells radiating from the central veins are separated from each other by the hepatic sinusoids, which connect the branches of the portal veins with the central intralobular veins. They also receive blood



FIG. 58.—Liver and gall bladder. (×75.)

from branches of the hepatic artery. The network of reticular fibers which surrounds the sinusoids is a continuation of the fibers of the capsule of Glisson.

The sinusoids are lined by two kinds of cells: the undifferentiated reticular cells, possessing small, dark staining, elongated nuclei, and the stellate cells of Kupffer, containing large oval nuclei. The cells of Kupffer are phagocytic and often contain pigment granules.

The liver cells are large polygonal cells with large round nuclei. The presence of two nuclei in a cell is frequent. Mitosis is rarely seen in the normal liver of an adult animal. The cytoplasm is extremely variable in appearance, depending on the functional state and the amount of glycogen or fat in the cell. The cell outline is often indistinct.

The bile canaliculi are present between adjacent liver cells and require special methods for demonstration. The canaliculi of one liver cell cord

receive short lateral branches from adjoining cords. These collect into the interlobular bile ducts, which are lined by cuboidal epithelial cells and are in proximity to the branches of the portal vein. As the tributaries of each lobe come together they form the hepatic duct. The common bile duct, formed by the hepatic and cystic ducts, also receives a branch of the pancreatic duct before entering the duodenum. The common bile duct is lined by tall columnar epithelial cells.

The gall bladder.—The gall bladder is attached to the posterior surface of the median lobe of the liver. It is lined by simple columnar epithelial cells which have cuticular borders. The thin lamina propria is composed of connective tissue. The mucous membrane forms many folds when the wall is not distended. The irregularly arranged smooth muscle fibers form a very thin layer. A delicate loose connective tissue sheet, part of the peritoneum, covers the free surface of the organ. The cystic duct connects the gall bladder with the common bile duct.

The pancreas.—The pancreas is a compound acinous gland, composed of many irregularly shaped lobes of varying size, distributed in the mesentery of the duodenum with its distal end in close proximity with the spleen. The lobes are covered by a thin connective tissue membrane which also divides them into lobules. The small secretory acini, which resemble those of the parotid gland, are composed of polyhedral shaped cells with dark staining round nuclei near the bases. Below and around the nucleus the cytoplasm stains deeply with basic stains, while above it the cytoplasm is light and contains granulations (zymogen). Mitotic figures are rare although they occur occasionally. Cytological variations exist due to the difference in the functional phase of the gland, and perhaps also due to the fact that the gland undergoes post mortem changes very quickly.

The secretion is collected in minute intercalated ducts lined by flat epithelial cells. As these ducts enter the acini they appear to be continuous with the centro-acinous cells. These cells are flattened, have relatively large, dark staining nuclei and a small amount of cytoplasm. The intralobar ducts are lined by cuboidal epithelial cells. One duct of the gland enters the common bile duct, while another enters the duodenum close to the entrance of the bile duct. At the entrance of the pancreatic duct into the duodenum small groups of pancreatic acini are usually present in the submucosa of the duodenum. In obese animals adipose cells are found in the interstitial tissue of the gland.

Irregularly distributed among the acini or the interstitial tissue are the pancreatic islands (of Langerhans), which function as glands of internal

secretion (Fig. 59). They are separated from the surrounding tissue by thin membranes. The cells of the islands are round, cuboidal or polyhedral in shape and form irregular cords. The round nuclei stain faintly. By special staining methods the presence of cytoplasmic granules can be demonstrated in the cells, showing differences in their staining reaction. Between the cords of cells, capillaries provide an intimate blood supply.

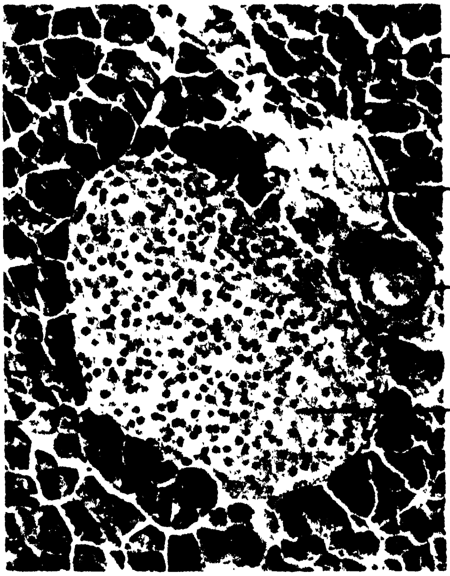


FIG. 59.—Pancreas with pancreatic island. (×200.)

RESPIRATORY SYSTEM

The larynx.—The larynx connects the pharynx with the trachea. Its walls contain cartilages covered by mucous membrane. The cartilages of the larynx are mostly hyaline; only in the epiglottis and in the vocal process of the arythenoid are elastic fibers present in the hyaline ground substance. The entrance of the larynx is guarded by the epiglottis which is attached by a stalk antero-ventrally to the thyroid cartilage. The lingual and the upper part of the laryngeal surface of the epiglottis and the aryo-epiglottic folds of the larynx are covered by stratified squamous epithelium. At the base of the epiglottis the epithelium changes from a stratified squamous type into a pseudostratified ciliated columnar type which extends over the entire surface of the larynx, except the true vocal cords.

The lamina propria of the epiglottis is continuous with and firmly attached to the perichondrium. The mucous membrane is rich in glands.

Small accumulations of lymphatic tissue are occasionally present near the base of the epiglottis. Projecting into the glottis are the false vocal cords, paired folds of the mucous membrane. Below these the true vocal cords arise. Between the false and the true vocal cords are lateral pouches, the ventricles of the larynx. The surface of the true vocal cords is covered by stratified squamous epithelium. The lamina propria consists of dense fibrous elastic tissue below which groups of striated muscle fibers are present. There are no glands in the mucous membrane of the true vocal cords. With the exception of these areas, glands are present throughout the entire length of the larynx. They consist of small groups of branched alveolar glands containing serous or mucous secreting acini and opening through short ducts into the lumen.

The cartilages of the larynx in old animals often show calcification.

The trachea and the main bronchi.—The trachea is continuous with the larynx. Its lumen is lined by pseudostratified ciliated columnar epithelium. The fibrous lamina propria is rich in blood vessels. The rigidity of the wall is due to the presence of hyaline cartilage rings. The rings are incomplete and one end of each ring is connected by smooth muscle fibers to the opposite end, forming the dorsal membranous wall of the trachea. The attachment of the musculature is on the outer (dorsal) side of the cartilage. The mucous membrane of the membranous wall is thrown into longitudinal folds. The perichondrium which surrounds each cartilage is continuous with the lamina propria. Only at the cephalic end of the trachea are there glands present in the lamina propria between the cartilage rings. Loose connective tissue forms the adventitia and attaches the trachea to the surrounding tissues.

The trachea divides into two main bronchi which are similar to it in structure. Here the cartilages form small irregular plates that completely surround the tube. Circular smooth muscle fibers complete the wall. Cartilage is not present in the walls of the bronchi beyond the point where they enter the lungs.

The lungs.—The thoracic cavity is lined by and divided into right and left pleural sacs by a very thin membrane, the pleura. The membranes of the two sacs meet in the median plane and form the mediastinal septum. The lungs are covered by the visceral pleura, a thin serous membrane composed of connective tissue containing collagenous and elastic fibers and covered by mesothelium. The left lung has one, the right four lobes. The inferior median lobe of the right lung is separated from the inferior lateral lobe by the inferior vena cava, and is enclosed in a separate pleural sac (Lauche in Jaffé, 56).

Each lobe receives, at its hilus, a branch from one of the two main bronchi. These branch repeatedly, gradually diminishing in size. The large bronchi of the lung are lined by pseudostratified ciliated columnar epithelial cells, among which goblet cells are present in varying number. The lamina propria consists of fibrous connective tissue containing elastic fibers. The mucous membrane forms longitudinal folds. Beneath this



FIG. 60.—Lung. (×100.)

membrane delicate smooth muscle fibers complete the wall. The bronchial tubes of the lung do not contain any cartilage. In the smaller bronchi the epithelial lining is simple columnar and ciliated. Short terminal bronchioles are formed by the division of the smaller bronchi and are lined by low columnar cells, cilia and goblet cells being absent.

The terminal bronchioles give rise to respiratory bronchioles, each of which in turn forms several alveolar ducts. Alveolar sacs, containing several alveoli, open from the alveolar ducts and form intercommunicating spaces separated from each other by thin walled septa in which capillaries anastomose. The respiratory bronchioles are lined by cuboidal epithelial cells, which are surrounded by connective tissue containing elastic and

collagenous fibers. Elastic and reticular fibers are present in the interalveolar septa. The cells which line the alveolar walls are the so-called "septal cells." They are large flat cells with oval nuclei and are closely attached to the walls of the capillary network. The entodermal or mesenchymal origin of these cells is still uncertain. The alveolar wall of the mouse contains a varying number of lymphocytes and occasionally granular leukocytes.

The lungs receive blood from the branches of the pulmonary arteries which follow the course of the bronchi. From these an arteriole passes to each alveolar duct and forms the network of capillaries in the walls of the alveoli. The pulmonary veins are formed by capillaries of the alveolar septa and of the pleura, and follow the course of the bronchi. The smaller bronchial arteries supply arterial blood to the wall of the bronchi and collect into the bronchial veins. The media of the walls of the veins in the lung are composed of cardiac muscle fibers (Fig. 60).

Urinary System

The kidney.—The kidney is a compound tubular gland composed of uriniferous tubules enclosed within a thin connective tissue capsule. A median section through the middle of the kidney shows a division into a cortical part containing mostly convoluted tubules, and a medullary part containing radially arranged straight tubules. The medulla is pyramidal in shape with the broad surface outward, and the apex ending in a single nipple-shaped dorsoventrally flattened papilla (Fig. 61). Columns of straight medullary tubules project part way into the cortex where they form the medullary rays.

The uriniferous tubules of the mouse are similar in structure to those of man, and for their detailed description the reader is referred to Maximow and Bloom (73).

It has been reported that in some mice the parietal or capsular epithelium of the capsule of Bowman consists partially or entirely of cuboidal epithelial cells (20, 42). Such capsules appear in greater number in the male than in the female animals (20).

The circulation of the mouse kidney is similar in general to the circulation in the human kidney (56). In the media of the glomerular arteries, in addition to the ordinary smooth muscle cells, larger, more afibrillar cells are present. These are similar to the cells described by Goormaghtigh (40) who states that they tend to accumulate in groups at the vascular poles of the glomeruli and form the "juxtaglomerular apparatus" (41).

The renal pelvis and ureter.—The funnel shaped pelvis surrounds the renal papilla. In its wide part the epithelial lining consists of a single layer of squamous cells which change gradually toward the narrow part first to polyhedral, then to two or three layered, still farther to four or five layered transitional type. At the wide part the lamina propria is very delicate and becomes better developed at the narrow part. An inner circular smooth muscle layer appears first about at the level where the epithelium becomes stratified, while still lower an outer longitudinal smooth muscle layer is also distinguishable. Outside of this, loose connective tissue containing many adipose cells surrounds the narrow end of the pelvis and the ureter which arises here (Fig. 61).

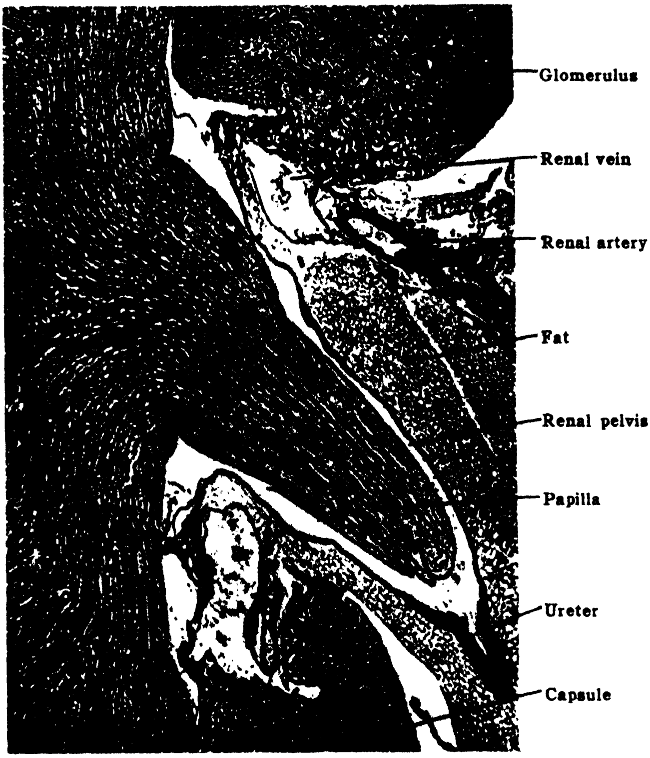


FIG. 61.—Kidney, region of pelvis and papilla. (×30.)

The ureter is a narrow tube which conducts the urine from the kidney to the bladder. Its wall is composed of transitional epithelium, a fibrous lamina propria, an inner circular and an outer longitudinal smooth muscle coat and the adventitia consisting of loose connective tissue and many

adipose cells. The mucous membrane forms low longitudinal folds. The ureters enter the dorsal wall of the neck of the bladder close to one another.

Bladder.—The bladder is lined by transitional epithelium consisting, when the organ is empty, of about four to five layers of cells. The fibrous lamina propria is rich in blood vessels. The mucous membrane is thrown into wide irregular folds and occasionally contains an aggregation of lymphocytes. When the bladder is in a distended condition the folds are absent and the epithelial lining is very thin. The smooth muscle coat consists of

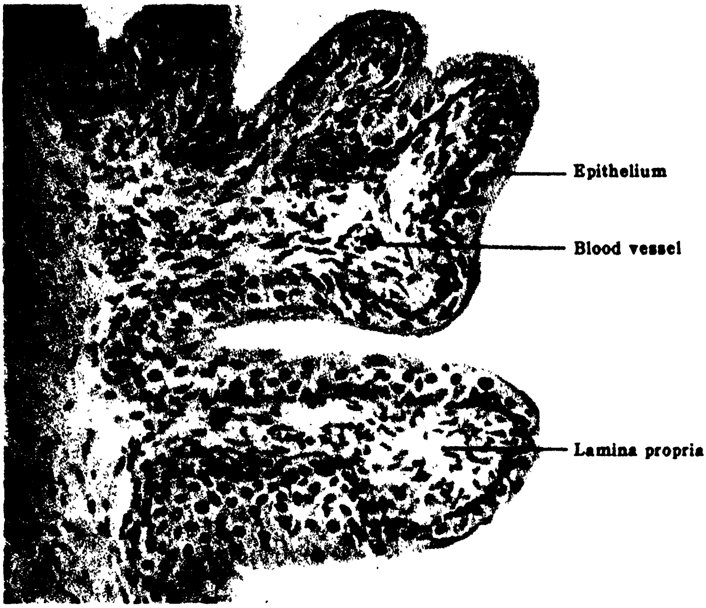


FIG. 62.—Bladder. (×200.)

irregular muscle bundles of varying size, separated from each other by considerable amounts of connective tissue (Fig. 62). At the neck of the bladder the direction of the muscle bundles is circular.

Female urethra.—The female urethra is a dorsoventrally slightly flattened tube which originates at the neck of the bladder and opens into the clitoral fossa. Near its origin the tube is lined by transitional epithelium which soon changes into stratified squamous type. The lamina propria is formed by loose connective tissue. The mucous membrane forms longitudinal folds. The epithelium forms invaginations which are continuous with gland tubules of the urethral glands. These glands are similar in structure to the urethral glands (of Littré) in the male. The circularly arranged smooth muscle fibers forming the outer wall are well developed. Near the

clitoris striated muscle fibers are also present. Loose connective tissue attaches the urethra to the ventral wall of the vagina.

Male Genital System

Figure 63 shows the dissected male genital system which includes the testes, a system of excretory ducts, the accessory glands, the urethra and the penis.

The testis and its excretory ducts.—The testis is a compound tubular gland in which the male sex cells, the spermatozoa, develop. The organ is covered by a fibrous connective tissue capsule, the tunica albuginea, from which, at the hilus, thin septa project into the gland and divide it into lobules. The lobules contain the convoluted seminiferous tubules. Arteries enter at the hilus, form a network on the inner surface of the tunica albuginea, penetrate with the septa, form a capillary network among the seminiferous tubules and collect into veins, the courses of which correspond with the courses of the arteries.

The tubules are lined by seminiferous epithelium resting on a basement membrane which, in turn, is surrounded by a thin layer of fibrous connective tissue. The interstitial stroma is rich in blood and lymph vessels and contains small groups of interstitial cells (of Leydig). The seminiferous epithelium is composed of two kinds of cells, the sustentacular Sertoli cells and the spermatogenic cells.

Under normal conditions the Sertoli cells lie near the basement membrane and are spaced at fairly regular intervals. The cells have large, oval, often indented nuclei and contain a compound nucleolus consisting of one central acidophil and two peripheral basophil bodies. When the cell is resting the nucleus is parallel with the wall of the tubule and the cell is polygonal in shape. When it is fulfilling its function of supporting the developing spermatogenic cells, the nucleus is perpendicular to the wall and the cell is pyramidal in shape. Under abnormal conditions, resulting in the degeneration of the seminiferous cells, the highly resistant Sertoli cells alone line the tubules and their cytoplasm forms a shapeless syncytium.

The primary spermatogenic cells, the spermatogonia, initiate spermatogenesis by repeated cell division. As the spermato- and spermiogenesis of the mouse do not differ in essentials from other mammals, for a detailed description the reader is referred to Maximow and Bloom's Textbook of Histology (73). Certain phases of spermatogenesis of the mouse are discussed by Cutright (21), Cox (19) Regaud (82) and Yocum (97).

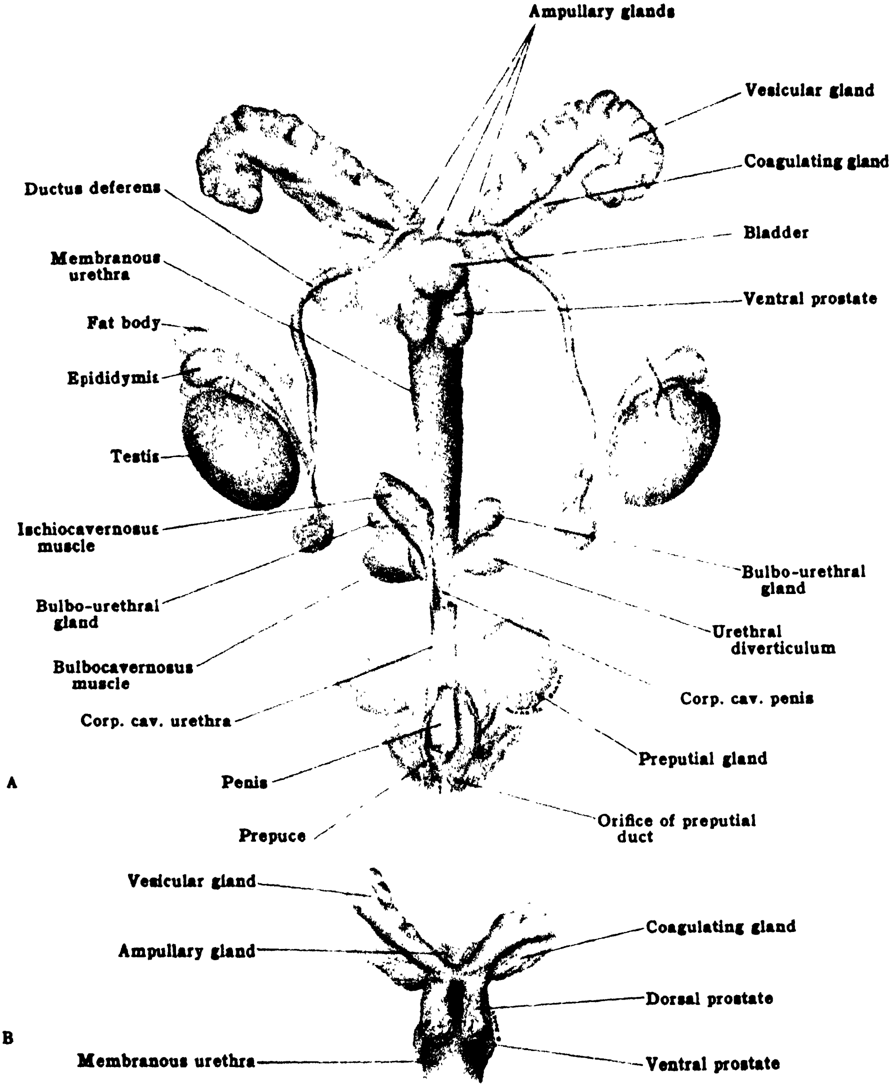


FIG. 63.—Drawings of the male genital system. A. Ventral view. The urethra is completely straightened. The left corpus cavernosum penis and bulbocavernosus muscle (right side of the drawing) are dissected, to show the urethral diverticulum, and the bulbo-urethral gland. On the right side (left side of the drawing) these structures are in normal position. The preputial sack is cut open. (×2¾.) B. Dorsal view of the cephalic end of the male urethra. (×2¾.)

The spermatozoon is composed of the head, the middle piece and the tail or flagellum. The head is flattened and hook-shaped, and ranges from .0068–.0102 mm. in length with a mean length of .0081 mm. The total length ranges from .1190–.1265 mm. with a mean length of .1227 mm. (Figures are based on 30 measurements made by Margaret Nickson.)

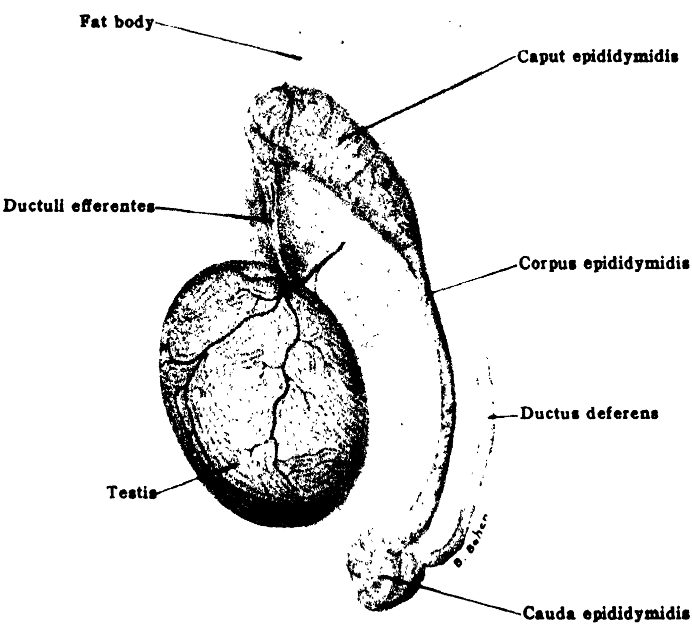


FIG. 64.—Drawing of the testis, efferent ducts, epididymis and ductus deferens. (×6.)

It was mentioned previously that the intertubular tissue contains small groups or cords of interstitial cells of Leydig. These cells have large round nuclei which contain one, or more often two, nucleoli and rather coarse chromatin granules. The cytoplasm stains intensely with eosin.

The excretory ducts of the testis include the rete testis, the efferent ducts, the epididymis which has three parts, the caput, corpus and cauda, and the ductus deferens (Fig. 64).

At the hilus the seminiferous tubules are collected into the network of an anastomosing system of canals, the rete testis, which is lined by simple, low cuboidal or at some places flattened epithelium. The network opens into a single lacuna which, outside the tunica albuginea, branches into as many parts as the number of efferent ducts. According to Benoit (6) this number

varies from three to seven. The number of efferent ducts in about 10 animals examined by us was three to five. The efferent ducts have two parts: beginning at the testis, in the first part the ducts have a short, straight, then convoluted course and are surrounded directly by the fat body of the testis; in the second part the ducts are highly convoluted and



FIG. 65.—Efferent duct joining the ductus epididymidis. (×200.)

are surrounded by a connective tissue capsule which becomes continuous with the capsule of the epididymis. The efferent ducts enter and form the first small segment in the caput of the epididymis. Our own observations are in agreement with those of Benoit (6) and Young (98) who found that the efferent ducts unite into a single duct which is in continuity with the duct of the epididymis (Fig. 65).

The lining of the efferent ducts is composed of alternating groups of tall and low columnar epithelial cells, which give the lumen a characteristic scalloped outline. The epithelial cells rest on a basement membrane,

below which a few circularly arranged smooth muscle fibers complete the wall.

The epididymis is covered throughout its entire length by a continuous sheath of fibrous connective tissue, which in the caput projects in and divides the convoluted tubules into seven to eight segments or lobules. As stated previously the first one of these lobules contains efferent ducts. In the second lobule the lumen of the ductus epididymidis is lined by very tall columnar epithelial cells. In most cells the oval nucleus is located in the lower third of the cell, while in some the nucleus is in a higher position. The cells possess non-motile stereocilia. Beginning at the third segment the epithelium lining the duct becomes considerably lower; the nuclei are at an even height and the duct is narrower. Approaching the cauda the duct becomes wider. On the inner surface of the basement membrane small round basal cells are present. The cross section of any part of the duct shows a very regular circular outline. A few circularly arranged smooth muscle fibers complete the wall.

As the ductus epididymidis leaves the cauda it becomes the ductus deferens. This duct is lined by tall columnar epithelium which at some places seems to be pseudostratified. The lamina propria is formed by fibrous connective tissue and the mucosa forms several prominent longitudinal folds. An inner circular and an outer longitudinal smooth muscle coat form a rather thick wall. Loose connective tissue, the adventitia, covers the duct (Fig. 66). Before entering the urethra the duct opens into the ampulla through a papilliferous projection. The epithelium changes suddenly, and the ampulla and its narrow neck, which connects it with the urethra, are lined by low columnar cells which have large, oval, deeply staining nuclei and small amounts of cytoplasm. The delicate lamina propria is surrounded by smooth muscle fibers. The mucous membrane forms many deep folds (Fig. 67).

The accessory glands.—Before giving the histological details of the accessory glands, a few general remarks are needed. The seminal vesicles are correctly referred to in the more recent literature as vesicular glands, because they do not contain or store spermatozoa but produce a secretion. The naming of the lobes of the prostate may cause confusion. There are three pairs of prostate glands, one pair of which is attached to the lesser curvatures of the vesicular glands. Because the secretion of this gland, according to Walker (95), produces coagulation when mixed with the secretion of the vesicular gland, it is often referred to as the coagulating gland, and this name will be adopted in this text. Rauther, (81) in a draw-

ing which has been frequently reproduced, illustrated the male urogenital system of the mouse and labelled this gland prostate I. The other two prostates are dorsally and ventrally located and will be designated in the text as dorsal and ventral prostates. Rauther referred to these as prostate II and III respectively. Occasionally the two lobes of the dorsal prostate are connected by a very small median lobe, but more often this lobe is



FIG. 66.—Ductus deferens. Fixed in Bouin's fluid. (×66½.)

absent. Around the base of the ductus deferens is a small group of glands whose ducts open into the ampulla. These are the ampullary glands (Fig. 63).

Figure 67 is a composite drawing of three adjacent sagittal sections of the urethra, slightly lateral to the midline, showing the entrance of some of the structures joining it. A short description of the more lateral sections is needed. The lateral wall of the cephalic end of the urethra is surrounded by the coagulating gland and the ventral and dorsal prostates. The dorsal prostate has many ducts, some of which are lateral to all the other ducts entering the urethra. Each coagulating gland has two ducts; they enter the dorsal wall of the neck of the bladder. The ventral prostate has several ducts which have a curved course caudad to the neck of the bladder, and enter the ventral wall of the urethra. The ductus deferens opens into a vestibule, the ampulla, which narrows down considerably before entering the urethra. Each vesicular gland has a rather wide duct and enters in

close proximity and dorsal to the neck of the ampulla. According to Disselhorst (28), the ducts of the vesicular gland and ductus deferens (neck of ampulla) join to form the ejaculatory duct before entering the urethra. In six animals examined by us the neck of the ampulla and the duct of the vesicular gland entered separately. However, variations exist and in a seventh animal the two ducts joined on the right side but entered separately

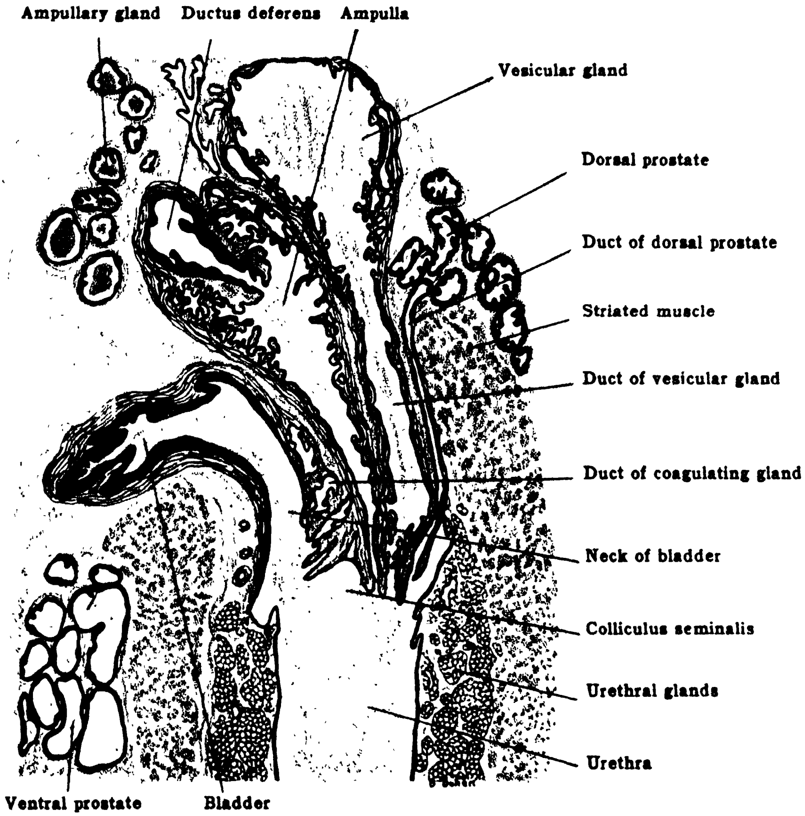


FIG. 67.—Cephalic end of the male urethra. A composite drawing of three adjacent sagittal sections. (×13.)

on the left side. The entrance of the neck of the ampulla and vesicular gland form a caudally diminishing elevation on the dorsal wall of the urethra, the colliculus seminalis.

Ampullary glands.—The ampullary glands are groups of branched tubular glands which open into the vestibular part of the ampulla. They are lined by low columnar cells having large oval nuclei. The lamina propria is very thin and the mucous membrane is thrown into many delicate, deep, longitudinal folds. The tubules are surrounded by a very thin layer

of circular smooth muscle fibers and held together in groups by a thin connective tissue membrane (Fig. 68). The color and apparent consistency of the secretions of the accessory glands in preparations stained with hematoxylin and eosin is characteristic and helpful in identifying them. The tubules of the ampullary glands contain an intensely red staining, dense,

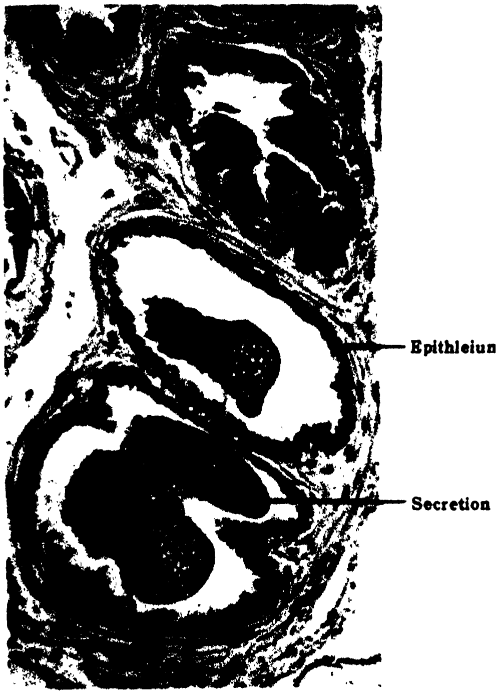


FIG. 68.—Ampullary gland. (×200.)

homogeneous secretion which has a tendency to shrink away from the epithelial lining and lie free in the lumina.

Vesicular glands.—The vesicular glands are long, narrow and curved at their tips. Internally there is a large, elongated cavity with medial alveolar outpocketings. The epithelial lining consists of a single layer of tall columnar cells having distinct cell outlines. The large oval nuclei are near the bases of the cells. The slightly basophilic cytoplasm contains dark secretion granules which are surrounded by lighter staining areas (halos) (Fig. 69). When the lumen is distended by secretion the epithelial cells are lower and do not contain secretion granules. The mucosa, especially on the side containing the alveolar outpocketings, is thrown into many fine, intricate folds. The gland is surrounded by smooth muscle fibers and

covered by a connective tissue sheath. The secretion, in sections stained with hematoxylin and eosin, is intensely red and has a tendency to crack and form parallel fissures.



FIG. 69.—Vesicular gland. (×175.)

Coagulating glands.—The branched, tubular coagulating glands are lined by simple columnar epithelial cells having round, centrally located nuclei and eosinophilic cytoplasm. The mucous membrane forms curved longitudinal folds, some of which project far into the lumen (Fig. 70). Even in the distended tubules some mucous folds are almost always



FIG. 70.—Coagulating gland. (×200.)

present. Each coagulating gland usually has two ducts which are lined by low columnar epithelial cells having deeply staining nuclei and slightly basophilic cytoplasm. Due to the folds present in the mucosa the ducts of these glands have a wavy lumen on section. The gland tubules are surrounded by a delicate layer of circular smooth muscle fibers and have a common connective tissue covering which attaches the gland to the lesser

curvature of the vesicular gland. The secretion is a faintly pink (H. E. stain) homogenous substance which forms fissures in sections of the larger tubules.

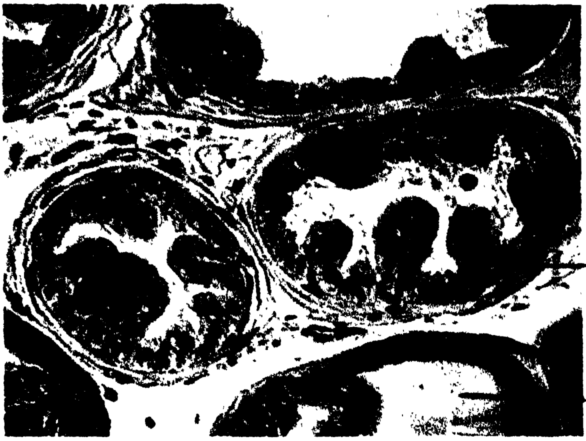


FIG. 71.—Dorsal prostate. (×200.)

Dorsal prostates.—The tubules of the dorsal prostates (Fig. 71) although considerably narrower, are very similar structurally to those of the coagulating glands. The color and apparent consistency of the secretion in section is



FIG. 72.—Ventral prostate. (×200.)

also similar; perhaps because the narrower tubules contain less secretion, the formation of fissures is rare. The gland has several ducts in which the mucous membrane, in contrast to the ducts of the coagulating gland, is free from folds.

Ventral prostates.—In the ventral prostates (Fig. 72) the gland tubules are lined by low columnar epithelium, having deeply staining spherical nuclei and slightly basophilic cytoplasm. The distended tubules do not contain mucous folds; in the smaller tubules folds are present. The secretion in a stained preparation shows a tendency to form round, pink staining globules of varying size. The gland tubules have a thin circular smooth muscle coat and are held together by a common connective tissue membrane.

The urethra, bulbo-urethral glands, penis and preputial glands.—The neck of the bladder is lined by diminishing rows of transitional epithelial cells. This changes into stratified squamous epithelium (two to three layers) lining the ventral wall of the urethra. The colliculus seminalis is covered by a continuation of the simple low columnar type of epithelium which lines the ducts entering on this projection. Similar epithelium lines the dorsal wall of the urethra. At a slightly lower level this also changes into stratified squamous epithelium which lines the membranous urethra throughout its entire length. Loose connective tissue forms the lamina propria, which is very rich in blood vessels and forms a framework for the urethral glands present in the mucosa (Fig. 67). These glands, as well as the thick layer of striated muscle fibers which surround them, appear on the dorsal wall near to its cephalic extremity and spread gradually caudally toward the ventral wall to form a complete sheath around the tube below the neck of the bladder. The urethral glands (of Littré) are composed of small groups of alveoli, the cells of which have oval nuclei near the base and cytoplasm containing basophilic secretion granules. Their short ducts, lined by cuboidal epithelial cells, open separately into the urethral lumen at different levels.

The root of the penis is attached to the pubic bone by the crura which are the terminal extensions of the corpora cavernosa penis. From the enlarged base of the crus penis, the ischeum, the ischio cavernosus muscle, arises and passes forward. The corpus cavernosum urethra proximally expands into the urethral bulb, over which extend the bulbo cavernosus muscle. The urethra forms paired lateral diverticula at the region of the bulb (Figs. 63 and 73). The lumen of each diverticulum shows variations in size and shape, depending on the amount of secretion present. It is lined by transitional epithelium, the apparent thickness depending on the dilated or relaxed condition. Below the epithelium, glands similar in structure to those of the membranous urethral wall are present. The diverticulum is surrounded by a fibrous membrane with circularly arranged, smooth muscle fibers as an inner layer. From here trabeculae composed of

fibrous connective tissue intermingled with smooth muscle fibers, project among the glands and form endothelial lined cavernous spaces. When these are distended, small groups of glands are widely separated from each other. When they are collapsed the glandular tissue appears compact. A heavy outer muscle sheath composed of striated fibers (m. bulbocavernosus) involves the diverticulum.



FIG. 73.—Sagittal section of the urethra showing the region of the bulb. Decalcified section of the pelvic region. (×30.)

Bulbo-urethral glands.—The paired bulbo-urethral glands (glands of Cowper) are retort-shaped organs composed of tubulo-alveolar glands. The body is partially covered by the musculus bulbocavernosus, while the tail runs throughout the muscles and its duct enters into the cephalic wall of the urethral diverticulum (Fig. 73). The tail is an aggregation of small gland lobules covered by a connective tissue membrane.

The body is surrounded by striated muscle fibers; inside this a very thin connective tissue membrane involves the gland and, projecting inward, forms the delicate inter-alveolar tissue. The tubules and alveoli are lined by tall columnar epithelial cells which have small, dark staining nuclei flattened against the bases of the cells. The cytoplasm stains pale blue with

hematoxylin-eosin stains. The cells rest on a well developed basement membrane. Occasionally the gland may be distended and contain large central cavities into which the tubules and acini open directly. In the lower part of the body a duct lined by cuboidal epithelium is present which anastomoses with the central ducts of the gland lobules of the tail. The gland lobules of the tail are composed of small alveoli lined by low columnar cells which have dark, round nuclei near the base and dark staining granular basophilic cytoplasm. Small groups of light staining cells similar to those present in the body are intermingled with these cells but in the part of the tail near the urethra these disappear and only the dark staining cells are present (Fig. 74). The long central duct of the lobule nearest to the urethra opens into the urethral diverticulum between the aggregation of the glands

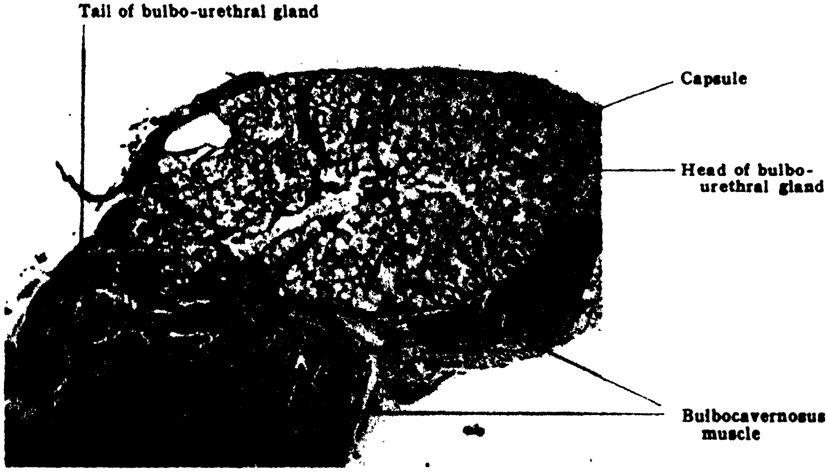


FIG. 74.—Bulbo-urethral gland. (×6.)

present in its lateral wall and the glands encircling the lumen of the muscular urethra (Fig. 73).

According to Hall (44) the secretion of Cowper's glands gives a positive reaction when stained specifically for mucin, while the glands of the urethra and the sinus give a negative reaction.

The penis.—The body of the penis consists of the thin corpus cavernosum urethrae and the two thick corpora cavernosa penis. The corpus cavernosum urethrae begins at the distal part of the diverticulum of the bulb, where it lies between the crura of the corpora cavernosa penis. It is composed of cavernous spaces surrounded by a layer of dense fibrous connective tissue, the tunica albuginea, the inner surface of which contains a layer of circular smooth muscle fibers. The cavernous spaces are formed by trabeculae con-

sisting of fibrous tissue, containing elastic fibers and a few smooth muscle fibers. The cavernous spaces are lined by endothelium. The urethral lumen, which occupies the center of the body, is lined by stratified columnar epithelium, which near the external orifice changes into stratified squamous. The fibrous lamina propria becomes continuous with the surrounding cavernous tissue. Glands are absent in the penial urethra. The proximal part of each corpus cavernosum penis is surrounded by its own tunica albuginea. Toward the distal part this becomes a narrow septum which finally disappears, and the cavernous spaces intercommunicate. The cavernous spaces are smaller near the periphery and larger toward the center. A small bone, the os penis, is found within the fibrous septum of the two corpora cavernosa penis and projects somewhat beyond the orifice of the penis.

The terminal end of the penis, the glans, lies within a protective chamber, the prepuce (or foreskin). The stratified squamous epithelium covering the glans forms low filiform papillae which make the surface slightly rough. Hair follicles are not present. The dense subcutaneous tissue contains some smooth muscle fibers. The mucous membrane which lines the preputial sack is a continuation of the covering of the glans (Fig. 63).

Preputial glands.—The large, flat, leaf-shaped preputial glands are homologous with the clitoral glands of the female (Fig. 63). They are large sebaceous glands surrounded by connective tissue capsule and consisting of rounded areas made up of large, flat, polyhedral epithelial cells with pale staining nuclei. The nuclei gradually disappear, and the cells degenerate forming a fatty secretion. Each gland has a long duct lined by stratified squamous epithelium which opens on the side of the tip of the prepuce. Near the orifice the epithelial cells of the duct and the subcutaneous tissue around it usually contain some pigment in non-albino animals.

Female Genital System

Figure 75 shows the dissected female genital organs which include the ovaries, the oviducts, the uterine horns, the corpus uteri, the cervix and the vagina. The fol'owing description of the attachment of the female genital system is based on the observations of Drahn (29) (Fig. 76). The ligamentum suspensorium ovarii which originates at the ovarian hilus extends anteriorly to the lateral surface of the kidney and attaches to the dorsal abdominal wall. This ligament contains some smooth muscles from which fibers project for a short distance into the ovarian capsule, increasing its elasticity and serving as constrictor muscles. The ligamentum ovarii proprium connects the hilus of the ovary to the cephalic end of the uterine

horn. This ligament is also rich in smooth muscle fibers which project into the mesotubarium and to the infundibular muscle. The infundibular muscle besides having connections with the above mentioned ligament has fibers projecting to the ovarian hilus. A narrow connection exists composed

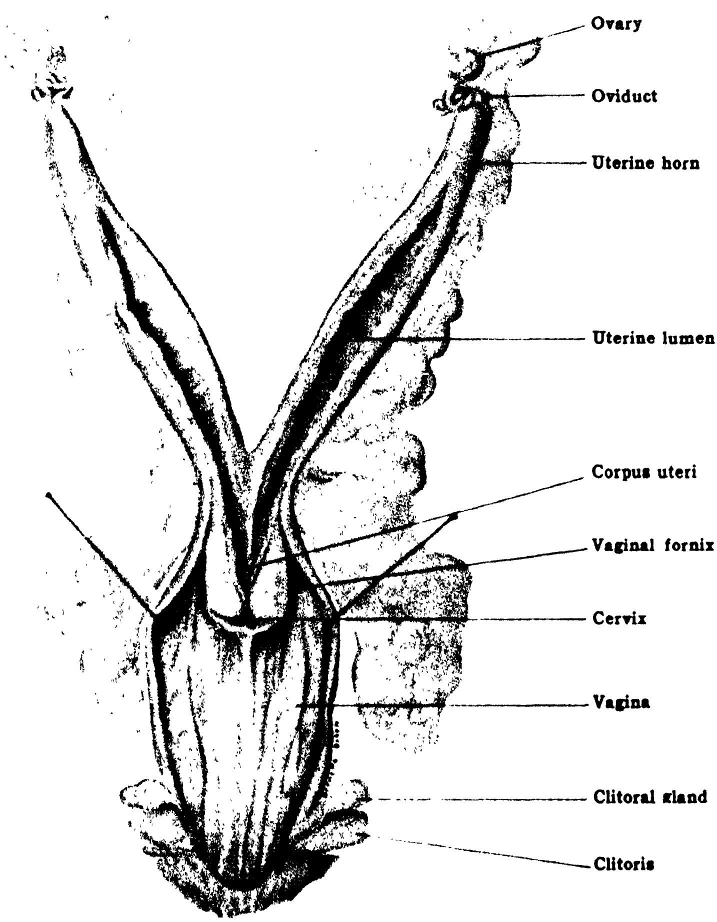


FIG. 75.—Drawing of the ventral aspect of the female genital system. The uterine horns as well as the corpus uteri, cervix and the vagina are cut open on the mid-ventral line. (×3.)

partly of connective tissue, partly of smooth muscle fibers between the tube and the ovarian capsule, and also with the ovary itself. Each uterine horn is attached to the dorsal wall by the mesometrium (broad ligament) which contains varying amounts of fat. Near the uterine horn the mesometrium contains longitudinal, smooth muscle fibers which are continuous with the

uterine musculature. Where the horns unite externally the mesometria join and end on the dorsal wall of the corpus.

The Ovary.—*The ovaries are paired glands in which the female sex* cells, the ova, develop. The free surface of the ovary is envolved in a thin transparent membrane, the ovarian capsule, which encloses the periovarian

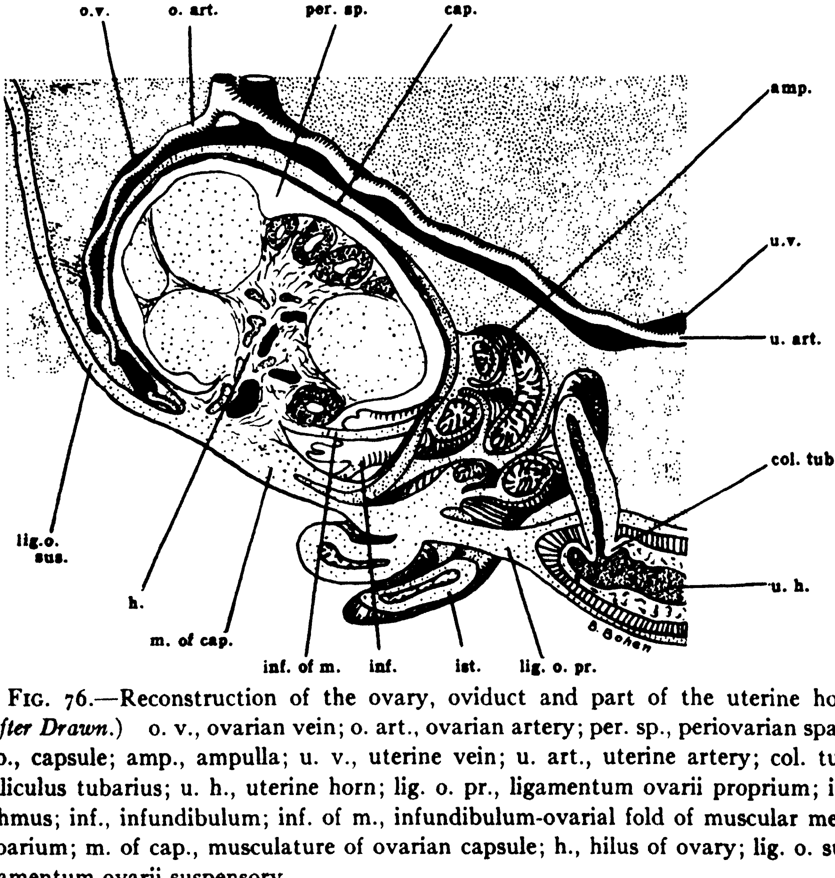


FIG. 76.—Reconstruction of the ovary, oviduct and part of the uterine horn. (*After Drawn.*) o. v., ovarian vein; o. art., ovarian artery; per. sp., periovarian space; cap., capsule; amp., ampulla; u. v., uterine vein; u. art., uterine artery; col. tub., colliculus tubarius; u. h., uterine horn; lig. o. pr., ligamentum ovarii proprium; ist., isthmus; inf., infundibulum; inf. of m., infundibulum-ovarial fold of muscular mesotubarium; m. of cap., musculature of ovarian capsule; h., hilus of ovary; lig. o. sus., ligamentum ovarii suspensory.

space. The ovarian capsule consists of a thin membrane of connective tissue covered on both surfaces by mesothelium. Small, blindly ending vestigial tubules of the Wolffian body, the epoophoron, may be present in or near the mesovarium. These are lined by cuboidal, often ciliated, epithelium and are surrounded by a thin circular smooth muscle wall.

A cross section of the ovary of an adult mouse (Fig. 76) shows an inner zone, the medulla (or zona vasculosa), and a surrounding outer zone, the cortex. Blood vessels enter and leave the organ at the hilus. They con-

tinue their course in the medulla which contains many large blood vessels separated from each other by a rather dense fibrous stroma. The free surface of the cortex is covered by a layer of cuboidal epithelial cells, the germinal epithelium, beneath which a thin layer of dense fibrous connective tissue forms the tunica albuginea. The primary follicles are immediately beneath the tunica albuginea, while those which are further developed are more deeply located.

A primary follicle consists of a large spherical cell, the primary oocyte, surrounded by a layer of squamous follicular cells. The nucleus of the oocyte is vesicular, contains small chromatin granules and a prominent nucleolus. Follicles which are somewhat further developed are lined by two or more layers of cuboidal follicular cells. Each such follicle contains a larger oocyte which is separated from the follicular cells by a transparent cell membrane, the zona pellucida. The connective tissue cells of the stroma are arranged concentrically around the follicle and form the theca folliculi. Around the larger follicles this layer has an inner part, the theca interna, which is rich in capillaries and contains large, loosely arranged cells, and an outer part, the theca externa, which contains concentrically arranged dense fibers.

In those follicles in which the development is still further progressed, small irregular spaces filled with a clear fluid, the primary liquor folliculi, appear among the follicular cells. These spaces gradually open into each other and form a single large fluid-filled cavity, the antrum. The antrum is lined by a stratified layer of follicular cells which in this position sometimes are called granulosa cells and which form the membrana granulosa. This membrane is thicker in the region where the oocyte, encircled by a group of follicular cells to form the cumulus oophorus, is attached. The follicular cells which immediately surround the zona pellucida are elongated and radially arranged. They are attached to the ovum by delicate cytoplasmic processes and form the corona radiata. The formation of the antrum and the increase in the amount of the liquor folliculi enlarges the follicle. Due to this expansion the follicle extends to the surface of the ovary and finally bulges out into the periovarian space. Such a follicle is called a mature vesicular or Graafian follicle. According to Brambell (11) the mean diameter of a ripe follicle in a section is about 530 μ. A single follicle may occasionally contain two or more ova.

Changes preceding and following ovulation.—The primary liquor folliculi becomes more viscid as estrus approaches. Preceding ovulation the secondary liquor folliculi is formed which is more fluid in character (85).

Small liquid filled cavities appear among the cells of the cumulus oophorus and the granulosa cells which line the antrum. These gradually detach the cumulus oophorus from the surrounding cells so that it floats free in the antrum (Fig. 77). In the meantime, in the nucleus of the ovum which lies near the surface of the cell, the nuclear membrane becomes faint, irregular and gradually disappears. The nucleolus also disappears, and the chroma-

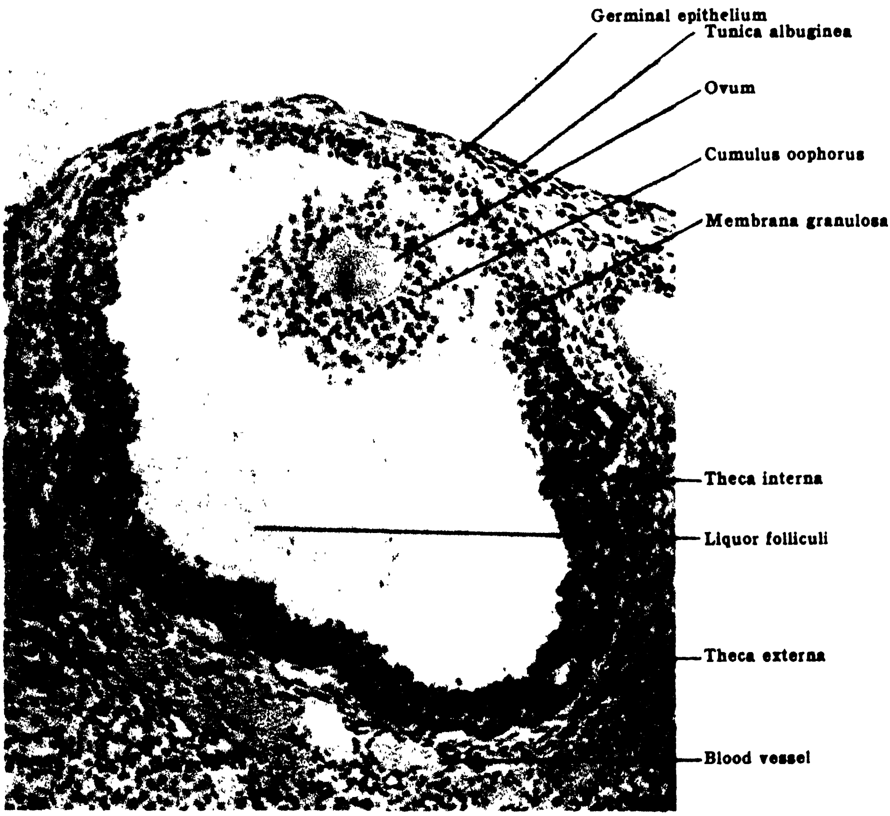


FIG. 77.—Mature ovarian follicle. (×150.)

tin granules form small dense chromosomes (20 tetrads). Delicate achromatic fibers form a rather narrow spindle and the chromosomes collect at the equatorial plane. There are no centrosomes or astral radiations. As the chromosomes begin to move toward the poles, 20 diads and a small amount of the cytoplasm are separated from the ovum by constriction and the first polar body is formed. It lies within the zona pellucida causing a bulging of its surface. The nuclear material of the ovum does not return to a resting condition. Instead preparations begin at once for the next division.

At this stage the ovum is free in the cavity of the mature follicle, surrounded by the cells of the cumulus oophorus. Ovulation occurs soon after this condition is established.

Parallel with the changes taking place in the ovum, changes also occur in the surrounding tissues. The large blood vessels of the medulla are engorged and the capillaries around the Graafian follicles show congestion. The thin wall of each follicle protruding into the periovarian space consists of flattened germinal epithelium, stretched tunica albuginea, the cells of which seem to be loosened by the congested capillaries, and one or two rows of granulosa cells. The opposite wall is considerably thicker, consisting of many layers of granulosa cells, and the theca interna which in section appears to project in waves into the follicle. When the thin wall ruptures, the ovum with the first polar body and the second polar spindle in the process of formation, surrounded by the cells of the cumulus oophorus, imbedded in liquor folliculi, are expelled into the periovarian capsule which, consequently, becomes distended. Several ova escape in a relatively short time interval, and due to the viscosity of the liquor folliculi they have a tendency to clump. They remain in the periovarian capsule only for a very short time, passing almost at once into the ampulla of the oviduct, which becomes distended. Fertilization takes place here and if spermatozoa enter the ova, the second divisions are completed. If fertilization does not take place, further development does not occur and the ova fragment and degenerate.

The ruptured follicle and the formation of the corpus leuteum.—After the bulging wall of the follicle has ruptured the tension is relieved and only a relatively small gap and cavity remain. The rupture does not cause bleeding, and normal ovulation is seldom followed by the formation of a hemorrhagic follicle. The free surface of the ruptured area contains enlarged capillaries, and the rich blood supply probably facilitates the rapid healing (Fig. 78). About 2 hours after ovulation the germinal epithelium and the tunica albuginea are united and the rupture is closed. From this time on the follicle is called the corpus luteum. In the young corpus luteum the theca externa keeps its circular outline, while the cells of the theca interna, which were beginning to project into the follicle even before ovulation, now penetrate still farther, carrying with them a network of developing capillaries, and are thus converted into vascular, radially arranged trabeculae providing support and blood supply to the granulosa cells. The trabeculae extend to the small inner cavity in which they form a loose network (Fig. 79). Later when the lutein cells are fully hypertrophied

this cavity disappears. At the beginning of this process mitotic figures are seen among the theca interna cells as well as among the granulosa cells.

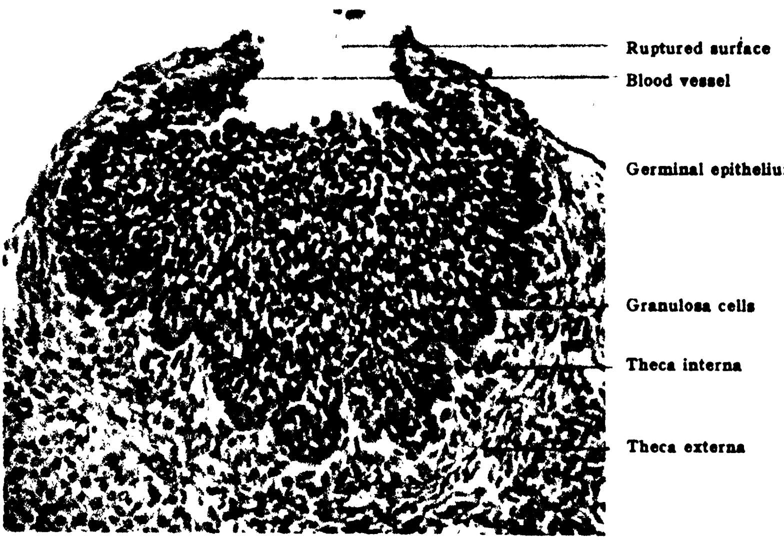


FIG. 78.—Ruptured follicle. (×62.)

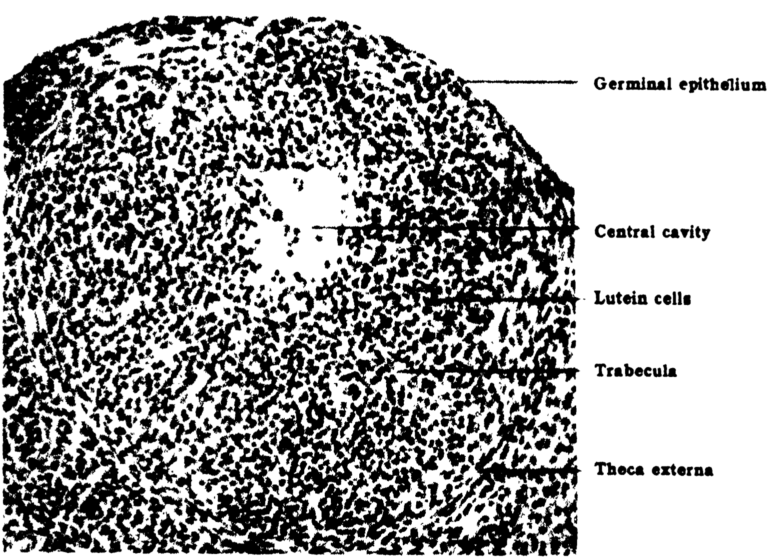


FIG. 79.—Young corpus luteum. (×50.)

Later the granulosa cells rapidly hypertrophy with resulting increase in size of the corpus luteum as a whole. While the small granulosa cells have oval

shaped nuclei with dark staining, coarse chromatin granules, surrounded by very little, slightly basophilic cytoplasm, the fully developed lutein cells are large and polyhedral with clear, slightly vacuolated eosinophilic cytoplasm and large round vesicular nuclei. The change from one type of cells to the other is gradual. The lutein cells are arranged in radial strands, surrounded by a network of sinusoidal capillaries. By the time they become established the central cavity has been obliterated.

Corpora lutea.—As ovulation usually occurs spontaneously in mice and the presence of one set of corpora does not inhibit ovulation, the ovaries may contain many sets of corpora lutea. According to Allen (2), "The most recent corpora lutea are easily distinguishable from the older ones by their blue color, the latter staining more heavily with eosin."

Deansley (26) conducted a detailed study of the fat accumulation in the corpora lutea of the mouse during the estrous cycle. (The fat granules were blackened by osmic acid preparation.) She found that in the corpus luteum of ovulation the fat and lipoid contents of the lutein cells gradually increase and that the granules become coarser as the next estrus approaches. At the time of the next ovulation the granules are less distinct, and 2 days after metestrus the cells contain hardly any fats or lipoids. Simultaneously the cells become smaller and cell outlines are less distinct. The corpora lutea of pseudo-pregnancy accumulate fat at a slower rate. Their cells are slightly larger, and the nuclei are smaller. In the corpus luteum of pregnancy, fats and lipoids are absent until about the 8th day after copulation. After that it contains finely distributed granules. The corpus enlarges until about the 13th day (mean diameter 976 μ, nearly 1 mm.). After this, little change takes place until about the 18th day when the corpus accumulates fat and a gradual shrinking starts. At parturition the outline of the corpus becomes indistinct and the fat and lipoid granules are coarser than at any time before, but not as irregular as at the end of estrus or pseudo-pregnancy. After parturition, although degeneration occurs, the body persists for a considerable time.

At the time of 10–12 days pregnancy, all the corpora lutea present in the ovary (except those of pregnancy) rapidly degenerate, forming fibrous masses containing large fat globules.

During lactation the corpora of pregnancy show a gradual shrinkage. During the first week the fats and lipoids show some increase, but this is followed by a decrease and a loss of the regular distribution. At the end of lactation diestrus (38 days after parturition) the corpora are fat free and have a mean diameter of 480 μ.

The corpora lutea of lactation are formed from follicles which ovulate post partum. They remain small and free from fat. The size of the cells is equal to those of the corpora of ovulation, but the nuclei are very small.

Atresia.—All of the follicles present in the ovaries do not mature and ovulate. Many of them undergo involution and gradual degeneration. This involution which is called atresia is a normal occurrence in the ovaries. Atresia may take place at any phase of the developing follicle. In the process of atresia of a primary follicle the ovum shrinks; it becomes wrinkled, the follicular cells become pyknotic and fragment, following which the surrounding stroma soon refills the space. In a larger follicle, after the degeneration of the ovum, the collapsed zona pellucida forms a hyaline clump which may persist for a considerable time. Occasionally the ovum shows pseudomaturation spindles or polar body formation. Atypical cell division of the ovum may lead to the formation of several cells of varying sizes enclosed in the zona pellucida. Such so-called parthenogenetic development is followed by degeneration. In some cases the partial degeneration of the follicular cells preceeds the degeneration of the ovum, and the latter is found "naked" in the middle of the follicle where it soon shows signs of karyorrhexis and cytolysis. Connective tissue cells and capillaries invade the follicle and replace the degenerated cells. The cells of the theca interna hypertrophy and form large polyhedral epithelioid cells, called theca lutein cells, which form the corpus luteum of atresia. Structurally such a corpus is similar to the normal corpus luteum, but usually contains some remains of the degenerated ovum or granulosa cells. These gradually shrink and are replaced by connective tissue. Strands of theca lutein cells may persist for a considerable time.

Occasionally (in virgin females quite often) a peculiar atresia takes place in a ripe follicle which fails to rupture. The granulosa cells do not degenerate, but hyertrophy and form a corpus luteum at the center of which the ovum is present. Sometimes the antrum of a follicle in the process of this type of atresia contains blood, and later is not filled in entirely by luteal cells but contains a loose connective tissue core. Gradually the ovum degenerates and hyalinization, progressing from the central area toward the periphery, sets in. The hyalinized corpus may persist as a round body for a considerable time, but finally shrinks and is gradually imbedded in the stroma.

In an Aschheim-Zondek test, after the injection into an immature female mouse of the urine of a pregnant woman, a similar type of atresia takes place. The follicles of the immature mouse ripen, pseudomaturation

spindles form and atypical, parthenogenetic development occurs. The antra fill with blood and later the granulosa cells hypertrophy to form corpora lutea atretica around the degenerating ova.

According to Engle (34), "A count of atretic follicles at four stages of the estrous cycle shows that there is a cyclic variation, both in the number of pseudomaturation spindles and in the total number of atretic follicles. The destruction is at its highest point during the first day of the diestrum, and at its lowest on the second day."

The oviduct.—The oviduct is often called the uterine tube or Fallopian tube. It is a narrow, coiled tube which connects the periovarian space with the uterus. The part nearest to the ovary, called the ampulla, ends in a funnel-shaped opening, the infundibulum (Fig. 76). The fringe-like edges of the ampulla, the fimbriae, extend into the periovarian space. The ampulla is continuous with the narrow isthmus, while the distal end of the oviduct, the intramural part, runs for a short distance within the wall of the uterus entering the lumen slightly eccentrically (pars interstitialis). Simple columnar epithelium lines the entire length of the lumen of the oviduct. In the ampulla these cells are tall, possess centrally located oval-shaped nuclei, strongly acidophilic cytoplasm and long motile cilia. Scattered among these cells are some non-ciliated club-shaped cells, which at certain phases of the estrous cycle protrude into the lumen (2). There is a short transitional zone between the ampulla and the isthmus, where ciliated and non-ciliated cells intermingle. The latter gradually prevail, and the rest of the oviduct is lined by low columnar cells without cilia.

The lamina propria consists of fibrous connective tissue. The mucous membrane of the ampulla forms narrow, high longitudinal folds. In the isthmus a few broad, low folds are present, while in the intramural part the folds are again somewhat higher. The muscular coat, which is formed by circularly arranged smooth muscle fibers, surrounds the mucous membrane. It is progressively better developed toward the intramural part. The tube is surrounded by a serous membrane which attaches it to the mesotubarium. As the intramural part of the oviduct enters eccentrically into the lumen of the uterus, it forms a papillary projection, the colliculus tubarius (Fig. 76). The projecting colliculus tubarius and encircling sulcus make difficult the injection of fluid into the oviduct from the uterus.

Uterus.—The uterus is composed of two horns which join to form an undivided caudal part, the corpus uteri (Fig. 75). The lumen of the uterine horn is lined by simple, columnar cells. Projecting down from the lumen are simple branched tubular uterine glands which are lined by low columnar

epithelium and have a spiral course deep in the mucosa. Occasionally they may penetrate into the muscular layer. The lamina propria consists of reticular tissue and contains many small polyhedral cells with relatively large, round nuclei. Lymphocytes are present and are especially numerous near the muscle wall. The mucosa (the epithelium, uterine glands and the



FIG. 80.—Transverse section of the uterine horns, corpus uteri, cervix and vagina. (×30.)

lamina propria) of the non-pregnant uterus is called the endometrium. It is well supplied with blood vessels. The endometrium is elevated into circular folds. The small polyhedral cells of the endometrium change during pregnancy into large epithelioid decidual cells.

The myometrium surrounding the mucous membrane consists of a compact ring of circularly arranged smooth muscle fibers, outside of which a layer of loose connective tissue containing large blood and lymph vessels

forms the stratum vasculosum. This in turn is surrounded by longitudinal, smooth muscle fibers. A serous membrane covers the horns and connects them with the broad ligaments.

As the horns come together the structure of the fused walls changes, losing first the longitudinal muscle layers and later the strata vasculosum. The circular muscle layers persist farther but disappear gradually and the two lumina are separated only by a wedge-shaped septum composed of longitudinal smooth muscle and connective tissue. Finally, the two lumina



FIG. 81.—Clitoral gland of a two weeks old mouse. (×25.)

join to form the single lumen of the corpus uteri. Laterally on each side of the corpus uteri the lumen of the vagina forms a deep fornix, while the mid-dorsal and mid-ventral walls of the corpus are fused with the wall of the vagina (Fig. 80). The corpus uteri opens into the vagina at the cervix, which is dorso-ventrally flattened. The epithelium of the corpus uteri consists of low cuboidal cells changing to stratified squamous at the cervix. A few shallow glands are present in the mucous membrane. The lamina propria is more fibrous and not as cellular as it is in the uterine horns.

Vagina, clitoris and clitoral glands.—The wide, dorso-ventrally flattened lumen of the vagina is lined by stratified squamous epithelium which undergoes cyclic changes during estrus. The lamina propria is formed of

vascular fibrous connective tissue. The mucous membrane which is devoid of glands forms longitudinal folds. The thin muscular coat contains some inner circular and outer longitudinal smooth muscle fibers, which are intermingled with considerable amounts of connective tissue. The wall is covered by loose connective tissue.

The vagina opens at the vulva. Immediately cephalad to the vaginal orifice the clitoris forms a small elevation. The subcutaneous tissue of the clitoris is rich in blood vessels, but does not contain any erectile tissue. The clitoris contains a small pouch, the clitoral fossa, which is lined by cornified stratified squamous epithelium. The urethra opens on the dorsal wall of this pouch, while ventro-laterally on each side open the ducts of the two clitoral glands (Fig. 75). These glands are considerably smaller, but similar in structure and position to the preputial glands of the male. The excretory ducts are lined by stratified squamous epithelium. The sac-like secretory alveoli are surrounded by a thin, connective tissue capsule, and consist of large pale staining, often vacuolated cells which like all sebaceous glands produce an oily secretion by cell degeneration. Each gland contains a single hair follicle with the hair shaft projecting into the duct (Fig. 81).

Mammary Glands

The mammary gland is a compound tubulo-alveolar gland. Mice have five glands on each side, three in the thoracic and two in the abdomino-inguinal region (Fig. 89). The gland undergoes several progressive and regressive changes during the lifetime of a breeding female. In the male a very small rudimentary duct system is present.

In the formation of the nipples all three layers of the epidermis take part (germinativum, granulosum and corneum). The skin covering the nipples is thickened and forms circular folds which allow for the stretching of the nipples at the time of nursing. In the formation of the duct system the stratum germinativum takes part. One main duct leads from each nipple into the subcutaneous fat pads and forms the collateral and terminal branches. The fat pads form a framework and seem to limit the growth of the fully developed glands. Each nipple with its main duct, collateral and terminal branches is a separate unit and is not in communication with the others.

The ducts are lined by cuboidal epithelial cells which have dark staining, oval nuclei and small amounts of cytoplasm. They are surrounded by circularly arranged connective tissue fibers. The connective tissue coat is

thicker around the main and primary ducts and gradually becomes thinner around the terminal ducts.

Before puberty the gland consists of long ducts which have few side branches. Shortly before puberty (between four to six weeks) more side branches develop and the distal terminal branches end in enlarged end-bulbs

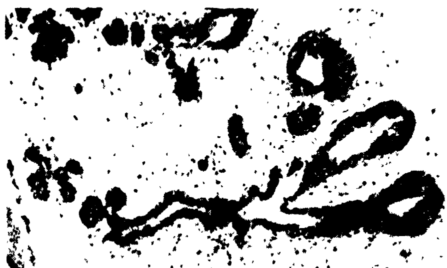


FIG. 82.—Mammary gland of an eight weeks old mouse showing rapidly growing end-bulbs. (×65.)

lined by several layers of cuboidal epithelial cells which contain mitotic figures (16, 35, 93) (Fig. 82). Increased mitotic activity and formation of the end-bulbs of the distal ducts at the approach of each estrus has been noted by several investigators.



FIG. 83.—Developing mammary gland on the 11-th day of pregnancy. (×200.)

A gradual increase in the epithelial elements of the gland by cell division is evident during the first part of pregnancy (Fig. 83). This increase reaches its peak at about the 11th–12th day, and results in the formation of alveoli. By the 14th to 15th day of pregnancy the alveolar system is well developed and mitosis is infrequent. Further development consists of an increase in size of the epithelial cells and an enlargement of the lumina of the ducts and alveoli. Secretory activity is established gradually, starting first in the alveoli proximal to the nipple and progressing distally. In the cytoplasm

small droplets of secretion appear which gradually fuse into large drops. The nuclei are pushed toward the base away from the lumina. At 17 to 19 days of pregnancy secretory activity is generally well established (Fig.

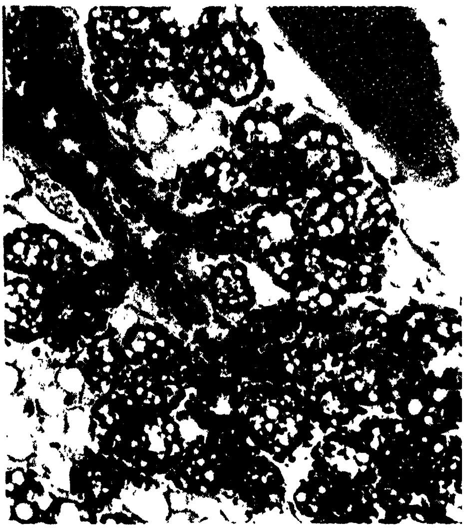


FIG. 84.—Mammary gland showing secretory activity near the end of pregnancy. (×200.)

84). (Parturition at 20 days.) Parallel with the glandular development there is an intensive development of blood vessels with which developing ducts and alveoli come in intimate contact. As the glandular parenchyma



FIG. 85.—Mammary gland on the 7-th day of lactation. (×100.)

occupies more and more space, the adipose cells of the fat pads rapidly disappear.

During lactation the ducts and alveoli are dilated and contain milk (Fig. 85). The original lobes and lobules of the fat pad supply the framework of the lactating gland, and adipose cells serve only to fill in the space

between the parenchymatous elements. The appearance of the epithelial cells of the alveoli is not uniform, indicating different phases of secretory activity. In some cells the nucleus is in the middle of the cell and the cytoplasm is homogeneous. In others the cytoplasm appears foamy or contains large protruding fat droplets.

If the litter is small and all the nipples are not suckled, some of the glands may undergo partial regression while others are still functioning (16, 35). The young mice usually suckle for about 21–23 days. The length of the suckling period depends somewhat on the size of the litter, large



FIG. 86.—Mammary gland 24 hours after lactation stopped. Lactation had continued for 22 days. (×100.)

litters usually suckling longer than small litters. About three weeks after parturition the glands begin to show signs of regression.

Twenty-four hours after suckling ceases, milk has accumulated in the ducts and alveoli, which are distended. Epithelial cells have become detached and are lying loose in the lumen. These degenerate, the cytoplasm becoming swollen and the pyknotic nuclei fragmenting (Fig. 86). In some epithelial cells the swollen cytoplasm forms globules which are discharged into the lumen, but the nuclei with small amounts of cytoplasm remain intact. The shrunken alveoli lose their close contact with the capillaries. The lack of blood supply hastens the process of regression. During this process the space between the shrinking alveoli is being filled by adipose cells. Some of these cells seem to develop from fibroblasts which are in

close proximity to capillaries. The nuclei of these fibroblasts become rounded and the cytoplasm increases in amount. Gradually fat accumulates in the cytoplasm and the nucleus is pushed to the periphery. The cells increase immensely in size during this change and adipose cells rapidly rebuild the fat pads. The collapsed alveoli form irregular clumps of cells which gradually undergo further degeneration. In the completely regressed, resting gland the lumina of the ducts are narrow, the epithelial cells lining them are small and darkly staining. The connective tissue sheath surrounding the ducts is increased in thickness. The glands remain in resting condi-

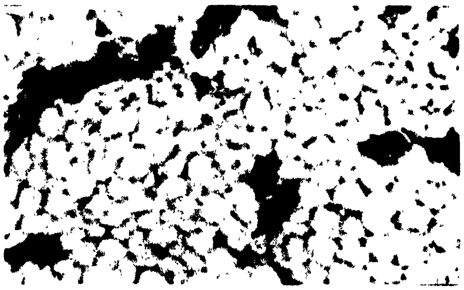


FIG. 87.—Mammary gland at resting stage. (×100.)

tion until the following pregnancy, when the described changes are repeated (Fig. 87).

In old females the glands undergo gradual involution. Part of each duct system atrophies and only the main ducts and a few secondary branches remain. The connective tissue surrounding the ducts becomes less cellular and more homogeneous.

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Chapter 4

SPONTANEOUS NEOPLASMS IN MICE

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INTRODUCTION

In our laboratory there have been over 20,000 mice that have developed spontaneous tumors. Many of these mice have developed multiple tumors involving different regions of the body. All of these cases have been carefully recorded and permanent preparations have been made of the vast majority of these tissues. Some have been studied as frozen sections.

As in the case of human neoplasms, practically all body regions have given rise to spontaneous new growths. Also, as with tumors in humans, the malignant nature of mouse neoplasms has been definitely established (23, 71). This has been a natural result of the enormous amount of research involving the investigation of all phases of mouse tumors since their value for this purpose was pointed out over forty years ago (42).

In this section spontaneous tumors will be treated as fully as the space permits. Most of the tumors described have occurred in the Roscoe B. Jackson Memorial Laboratory mice.

During the past ten years the staff of the laboratory has kept detailed records on large colonies of mice representing a considerable number of inbred stocks. One phase of the record-keeping included the collecting of detailed data on the incidence of spontaneous tumors. Complete autopsies were routinely performed and tissues were saved from all body regions which offered any suggestion of abnormal growth. Furthermore, no tumor experiments have been considered ready for publication until these tissues have been studied as to their histopathology. Our collection of tissues from mice which spontaneously developed tumors represents data from stocks that vary widely in their tumor incidences. Data on the high tumor strains show that in some stocks over 90 per cent of all breeding females living into the tumor age develop some form of neoplasm, e.g., the A and C_3H strains, while in the low tumor strains abnormal growths are rarely found. even in mice which have attained extreme senility, e.g., *Mus bactrianus.*

Naturally, the number of recorded mice with spontaneous tumors is no indication of the vast numbers of mice which have been employed by the staff, for the stocks vary so markedly from one to another in their population tumor incidence. However, inbreeding has been carried on to such a degree that, on the basis of previous observations within a stock, one can predict with considerable accuracy what types of tumors will probably occur, at what average age they will be found and in the case of certain types of growths, in what per cent of another large population these new growths will develop when one employs the same stock.

Definition and Characteristics of Tumors

A tumor is an autonomous new growth of tissue (Fig. 88). Also, tumors are atypical growths with atypical structure, apparently of independent origin. They exhibit no useful function, are without limit to growth and, if uninterrupted, can result in the destruction of the host. These growths arise either from embryonic cell rests or from the body cells of the host. They start as a localized disease involving a few cells and progressively increase in size by cell division.

Some masses of cells may grow by *expansion.* This will result in the formation of a connective tissue capsule due to the pressure of the growth on the surrounding supporting cells. Others may grow by *infiltration*, spread along tissue spaces and lymphatics and may be found at some distance from the spontaneous tumor. This is a more malignant type than the former. Another form of growth combining the two above types is called *interlocking.* The second and third types of growth are the most difficult to remove by

operation and unless complete removal is effected recurrence takes place, often accompanied by an increase in rate of growth.

As long as neoplasms are in contact with serum they acquire an independence of growth. Spread, or *metastasis*, through serum is the greatest danger to the life of the host and one of the chief factors of malignancy. Within the host metastases may be lymphogenous,* hemogenous, implantation or transplantation. New colonies of similar tumor cells are established at a distance from the primary tumor, and these in turn may spread to other locations until a generalized involvement of the entire host organism results.

Tumor cells are parenchymatous neoplastic cells of connective tissue or epithelial descent. The connective tissue tumors form their own stroma



FIG. 88.—A spontaneous mammary gland carcinoma (×½).

and blood vessels, while in the epithelial tumors the stroma and blood vessels are from the surrounding tissues, with the result that in the latter tumors the vascular supply is abnormal, atypical and more sinusoidal than in normal tissue. There are no new-formed lymphatics in tumors, and it is generally believed that there are no nervous system attachments (21, 31, 38).

A mouse frequently appears in excellent health when a tumor is small but grossly detectable. As the tumor increases in size, it places increasing nutritional demands upon the host and at the same time there frequently are metabolic changes due to infiltration of, or pressure upon, vital organs. This is accompanied by degenerative changes in the tumor resulting from the faulty blood supply, so that parts of the tumor become necrotic and their waste products find their way into the blood stream. The mouse will develop progressive signs of general ill health with loss of weight and evidence of malnutrition, until in the late stages there is a ruffling of the hair, weakness, lowered body temperature and a tendency to kyphosis which gives

* Rare in mice for lymphatics are so small and delicate that they are easily occluded (44).

the animal a shrunken appearance. With certain neoplasia there also develop respiratory difficulties and in some cases marked edema. There is a great variation in the speed at which different tumors grow, so that in some cases the mouse may die from other causes without having been greatly inconvenienced by a slow growing neoplasm.

A vast amount of work has been done on the etiology but the exact cause of cancer remains unknown (9, 11, 12, 37, 71). It is known that chronic inflammation of either intrinsic or extrinsic origin may accompany the onset, but chronic inflammation of itself is not enough to change normal body cells into outlaw cells the chief function of which is unrestrained growth and which exhibit a total disregard for normal tissue boundaries. Neoplasms may develop from any cell, organ or tissue of the body which is capable of growth.

To summarize the characteristics of a neoplasm, we can say that it is an autonomous new growth of undetermined origin which starts locally, serves no useful function, may invade the adjacent tissues and even be transferred to distant body regions of the host, grows progressively and, if uninterrupted, eventually results in the death of the host.

Tumors may be either *malignant* or *benign*. The benign forms are homeotypic in structure, relatively slow growing, grow by expansion and are encapsulated so that they do not infiltrate and do not metastasize. This makes complete removal possible, in which case they will not recur. On the other hand, the malignant forms are heterotypic in structure and possess no capsules, so that infiltration and metastases are the most important characteristics of this group. A benign tumor may develop into a malignant form showing infiltration and metastasis. This is fairly frequently seen in carcinoma of the breast in mice, where a small, partially encapsulated adenoma may be continuous with an adenocarcinoma.

Classification of Tumors

There are several methods of classification of tumors (11, 12), but the histological structure offers the simplest means, especially with small experimental mammals such as the mouse. A tumor receives a name according to the tissue which it most resembles. However, this applies best to only the simple and benign tumors since many malignant forms do not resemble any normal tissue. The terms *sarcoma* and *carcinoma*, therefore, have been employed to designate the two main groups of the malignant neoplasms (11). A sarcoma is a malignant tumor composed of cells of the connective tissue type. It is formed on the connective tissue plan, developing its own stroma

and blood vessels so that the stroma and blood vessels are in intimate contact with the tumor cells. The main object in this classification is to separate a large group of malignant tumors from the carcinomas; however, this method ignores certain embryological considerations. A carcinoma is a malignant tumor originating from the epithelial cells of the skin, the mucosa or their derivatives. In general, sarcomas present a smooth, rounded contour, while the carcinomas appear less uniform in consistency and frequently give a nodular appearance.

HISTOLOGICAL CLASSIFICATION OF MOUSE TUMORS

I. **Connective tissue** (types 1 through 7 are benign).
  1. Fibroma—connective tissue origin.
  2. Myxoma*—mucous connective tissue origin.
  3. Lipoma—fat tissue origin.
  4. Chordoma*—Chorda dorsalis tissue origin.
  5. Chondroma—cartilage tissue origin.
  6. Osteoma—bone tissue origin.
  7. Angioma
    a. Hemangiona—blood vessel origin.
    b. Lymphangioma—lymph vessel origin.
  8. Sarcoma—a malignant cellular tumor composed of anaplastic tissue of any of the above types 1 through 7.
    a. Fibrosarcoma.
    b. Neurogenic sarcoma.
    c. Myxosarcoma.
    d. Liposarcoma.
    e. Chondrosarcoma.
    f. Osteogenic sarcoma.
    g. Angio-endothelioma.
    h. Round cell sarcoma.
      (1) Lymphocytoma.
      (2) Myelocytoma.
      (3) Monocytoma.

II. **Muscle tissue.**
  1. Myoma (benign).
    a. Leiomyoma—smooth muscle tissue origin.
    b. Rhabdomyoma—striated muscle tissue origin.

* As yet not reported in mice.

2. Myosarcoma (malignant).
   a. Leiomyosarcoma.
   b. Rhabdomyosarcoma.

III. Elements of the nervous system.

1. Neuroma—nerve fiber origin.
2. Neuroganglioma—nerve fiber and ganglion cell origin.
3. Glioma and medulloblastoma—neuroglia tissue origin.
4. Neuro-epithelioma—from neuro-epithelium.

IV. Tumors of pigment cells.

1. Melanoma.
2. Malignant melanoma.

V. Endothelium.

1. Endothelioma—blood and lymph vessel endothelium origin.

VI. Epithelial tissue (pavement and glandular).

1. Papilloma—a benign tumor of pavement epithelium with supporting tissue in a normal arrangement.
2. Adenoma—a benign tumor of glandular epithelium with supporting tissue in normal arrangement.
3. Epithelioma (epidermoid carcinoma, squamous cell carcinoma, acanthoma)—a malignant tumor of pavement epithelium in atypical arrangement.
4. Carcinoma—a malignant tumor of glandular epithelium in atypical arrangement.

VII. Complex tissue tumors.

1. Simple mixed tumor—composed of more than one type of neoplastic tissue and named according to composition—carcinosarcoma, adenofibroma, fibro-adenoma, etc. The predominating type is named last.
2. Teratoma—composed of tissues and organs of one, two or three germinal layers, such as monodermal, bidermal or tridermal types.
3. Embryoma—composed of tissues from three germinal layers in a more or less orderly imitation of the fetus.

VIII. Cysts, not neoplasms, but related to them and in mice often mistaken for them by gross observation.

Some types of tumors have been reported in the literature as rare. However, our experience has been that the frequency of spontaneous tumors of any particular type is dependent, to a certain extent, upon the lines of inbred mice under observation. Had our observations been limited to the C57 black stock and other lines low in epithelial tumors of the mammary glands

such as C57 leaden, black hairless and *Mus bactrianus*, our impression would have been that mammary tumors are rare. The reverse would have been true had we been using only such stocks as the A albino, the C_3H, the dba and similar lines with a high incidence of spontaneous breast cancer. To give more concrete examples, the A albino line is high in the incidence of both lung and mammary cancer, while lung cancer is uncommon in most of our other stocks. On the other hand, the ce (extreme dilution) stock has an abnormally high number of tumors of the ovary, while most of the melanomas

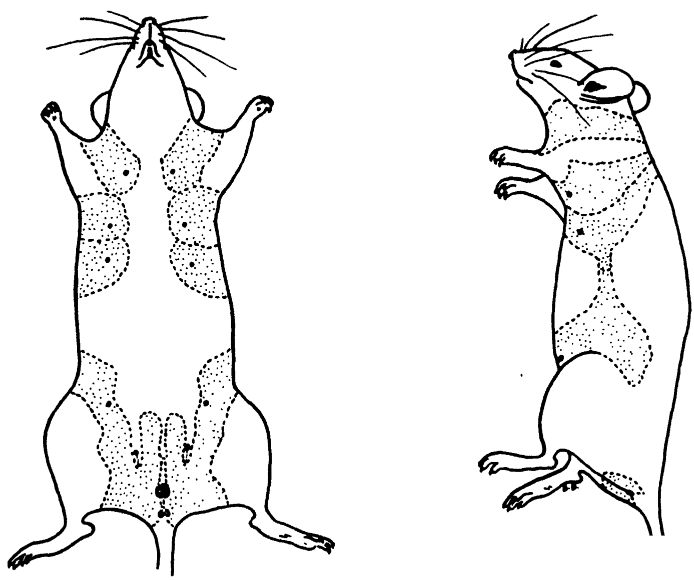


FIG. 89.—A diagrammatic drawing of the maximum extent of the mammary system of the mouse shown in the ventral and lateral aspects. The large black dots represent the nipples and the stippled areas the mammary glands.

have been observed in the dba (dilute brown) stock, and tumors of all types are rare in *Mus bactrianus*. Certain tumors will probably always be considered uncommon, but as an increasing number of inbred stocks are developed and studied, we shall be able to find certain lines of mice which will be of exceptional value in advancing our present knowledge of the more rare forms of spontaneous neoplasms.

Tumors of the Mammary Region

Since the majority of investigators, especially the pioneers, working with spontaneous tumors in mice confined their studies mainly to the most available forms of neoplasms, they investigated chiefly those outside the body cavities and thus much of the work has been done with tumors of the mam-

mary region. The normal mammary glands have been fully discussed under the section on histology, but for convenience the distribution of the mammae can be briefly reported here. There are five pairs of glands arranged symmetrically along the ventral surface of the mouse (Fig. 89). This rather extensive distribution of the mammary glands is referred to here as the mammary line and its branches.

Because of the accessibility to observation the subcutaneous tumors are probably better known than those of other body parts, and since the majority of tumors observed are in or near the mammary region, they must be studied histologically to determine their true nature.

Mammary region tumors have been divided into two main groups in an attempt to include all the types of tumors which occur in the region of the mammary line and its branches. First are those tumors which originate from the mammary gland proper while the second group comprises all other tumors in this same location, but not arising from the mammary gland or its supporting stroma. This grouping is intended to cover the masses which, by gross observation, might be mistaken for tumors of mammary gland origin as well as those which arise from the gland itself.

Classification of Tumors in or Near the Mammary Glands

I. Tumors originating from the mammary gland proper.

A. Benign tumors.

1. Simple adenoma.
2. Polylocular cyst adenoma.
3. Papillary cyst adenoma.
4. Fibro-adenoma (adenofibroma).

B. Malignant tumors.

1. Adenocarcinoma—definite evidence of mammary gland origin predominates the histological picture.
    - *a.* Simple adenocarcinoma.
    - *b.* Adenocarcinoma (variable type).
    - *c.* Papillary cyst adenocarcinoma.
    - *d.* Intracanalicular adenocarcinoma.
    - *e.* Macroglandular adenocarcinoma.
2. Carcinoma simplex—little evidence of definite gland formation.
    - *a.* Round cell or medullary.
    - *b.* Spindle cell.
3. Carcinosarcoma—originating from both the mammary gland epithelium and the stromal connective tissue.
4. Fibrosarcoma—originating from the mammary gland stroma.

II. Tumors originating in or near the mammary line and its branches but not arising from mammary glands or their stroma.

A. Benign tumors.
1. Fibroma.
2. Chondroma.
3. Osteoma.
4. Lipoma.
5. Angioma.
   a. Lymphangioma.
   b. Hemangioma.

B. Malignant tumors.
1. Fibrosarcoma.
2. Melanoma.
3. Osteogenic sarcoma.
4. Rhabdomyosarcoma.
5. Carcinomas of skin appendage.
6. Round cell sarcoma—axillary and inguinal lymph nodes.
7. Endothelioma.
   a. Hemangio-endothelioma.
   b. Lymphangio-endothelioma.

There are also non-neoplastic masses that grossly resemble true neoplasms and these will be merely listed.

1. Cysts.
   a. Mammary duct cysts.
   b. Skin cysts.
   c. Hygromas—thin-walled, endothelial-lined, cysts filled with lymph. Seen in C57 black stock.
2. Chronic inflammation.
   a. Subcutaneous in general.
   b. Chronic mastitis—fibrosis and lymphoid infiltration of the mammary gland, usually not accompanied by cyst formation in the mice.
3. Lymphoid hyperplasia.

Adenomas of the Mammary Glands

The tumors within this benign group have certain characteristics in common. Grossly they are comparatively small, frequently indistinguishable from soft non-hemorrhagic carcinomas, and sometimes they appear to be soft, cystic, translucent masses. As seen under the microscope they have

a connective tissue capsule which may be thick in some places and difficult to follow in others. The capsule is not invaded by the tumor cells. The epithelial cells are arranged as gland-like structures which are easily recognized as mammary gland in origin. These structures may vary considerably in size and arrangement, but they are always lined by a single layer of fairly uniform, usually small, and relatively inactive epithelial cells. Under these

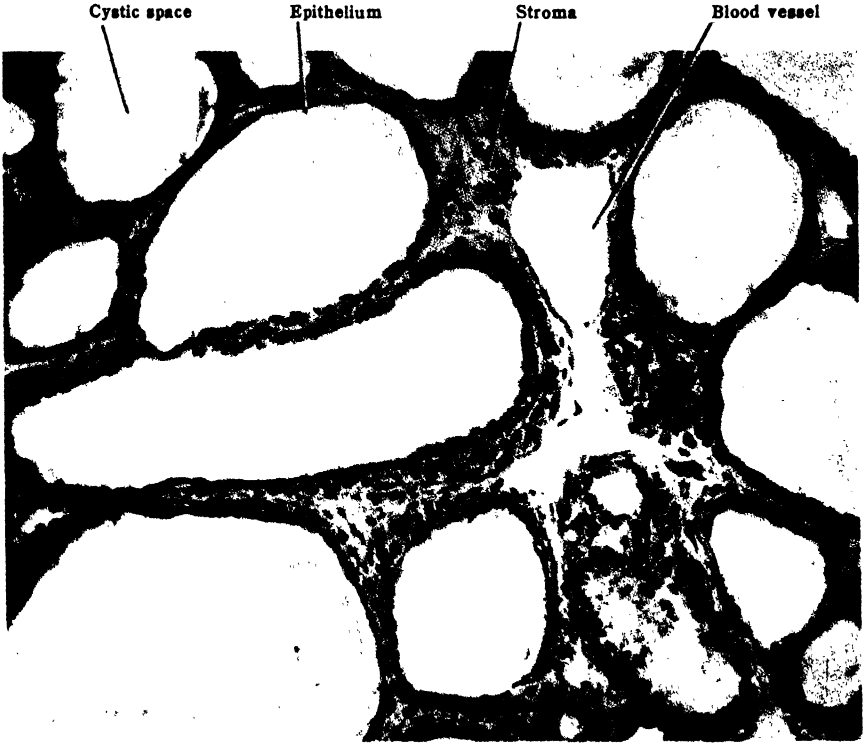


FIG. 90.—Simple adenoma of the mammary gland (X200).

cells the basement membrane remains intact and around this there are seen a few small, thread-like, wavy, spindle-shaped connective tissue cells.

Simple adenoma of the mammary gland.—This is not commonly seen as such, but it is not rare to observe the remains of this type in direct continuity with carcinoma of the mammary gland. These adenomas contain gland-like arrangements of the mammary epithelial cells (Fig. 90). They range from small abortive structures which appear to be attempts at gland formation to large, round or oval, dilated cyst-like spaces containing more or less eosinophilic amorphous material. The lining cells are arranged in a single, smooth, orderly layer. Generally they are cuboidal and uniform in size, shape and

staining properties. The basement membrane is well preserved. These cells do not differ strikingly from inactive, normal mammary gland epithelial cells. In some cases the lining epithelial cells of the cysts range from the small cuboidal type with moderately deeply staining nuclei and scant cytoplasm to fairly large, oval cells which bulge into the cyst cavity. These latter cells have large, pale, centrally placed oval nuclei which contain



FIG. 91.—Polylocular cystadenoma of the mammary gland showing intercommunicating cysts (×200).

scattered chromatin granules and multiple nucleoli. The cytoplasm is eosinophilic, uniformly staining and fairly abundant. In simple adenomas the stroma does not bulge into the epithelial-lined cavities. These gland-like arrangements may have foci that are uniformly large or small but are usually distributed so that the whole range can easily be found in a single low power field. Mitoses are seen but are infrequent.

The stroma is rather loose in the foci where the gland-like structures are most widely separated and contains scattered strands of connective tissue. Beneath the basement membrane of each of the gland structures the connec-

tive tissue is more compact and may consist of one to several layers. These connective tissue cells are wavy and thread-like in appearance, have centrally placed spindle-shaped nuclei and possess pale eosinophilic cytoplasm. The nuclei are moderately pale, with somewhat evenly distributed small chromatin granules. It is beneath this compact layer of connective tissue that the loose stroma is found when present. Where the adenomatous

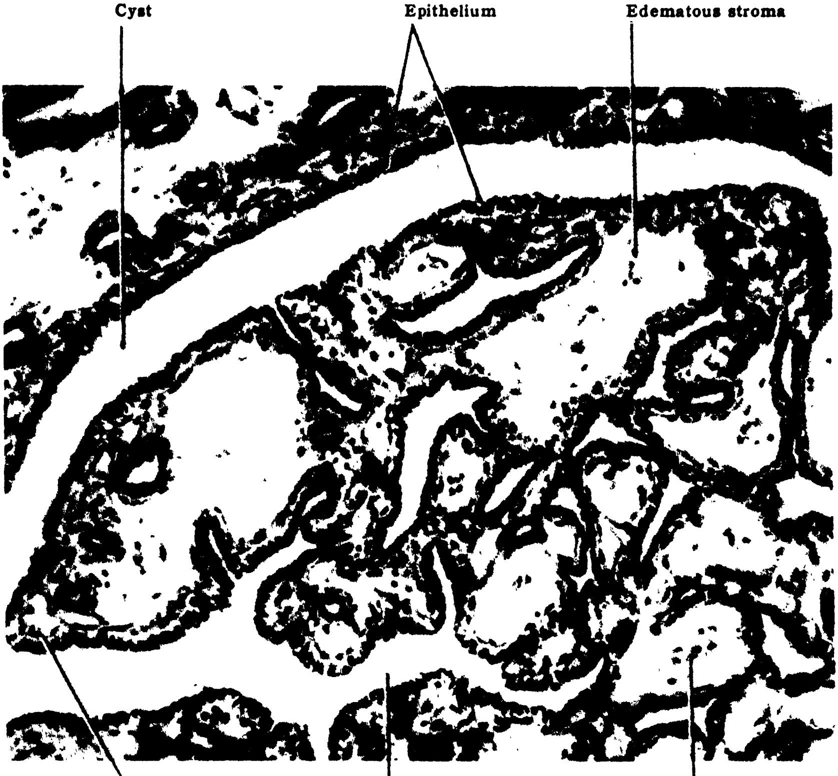


FIG. 92.—Papillary cystadenoma of the mammary gland (×200).

structures are most compact the adjacent epithelial layers of different glands may be in very close relationship with only narrow septa of stroma between them. Large and small, irregularly shaped, thin-walled, endothelial lined blood spaces are scattered throughout the stoma, most prominently in the looser foci. The capsule is composed of a dense connective tissue layer which may vary somewhat in thickness so that in some foci it is difficult to distinguish.

Polylocular cyst adenoma of the mammary gland.—This type shows many large and some small irregularly shaped, frequently intercommunicat-

ing, epithelial lined cysts (Fig. 91). These lining cells form a single orderly layer, are low cuboidal, closely packed and uniform in size, shape and staining properties. They have scant cytoplasm and somewhat rounded, deeply staining nuclei. Mitoses are infrequent. The walls of the cysts are not smooth as in simple adenoma, but have an irregular wavy appearance without the formation of papillary ingrowths.

The stroma is composed of coarse and fine, wavy, eosinophilic non-nucleated fibrils, throughout which are scattered thread-like connective

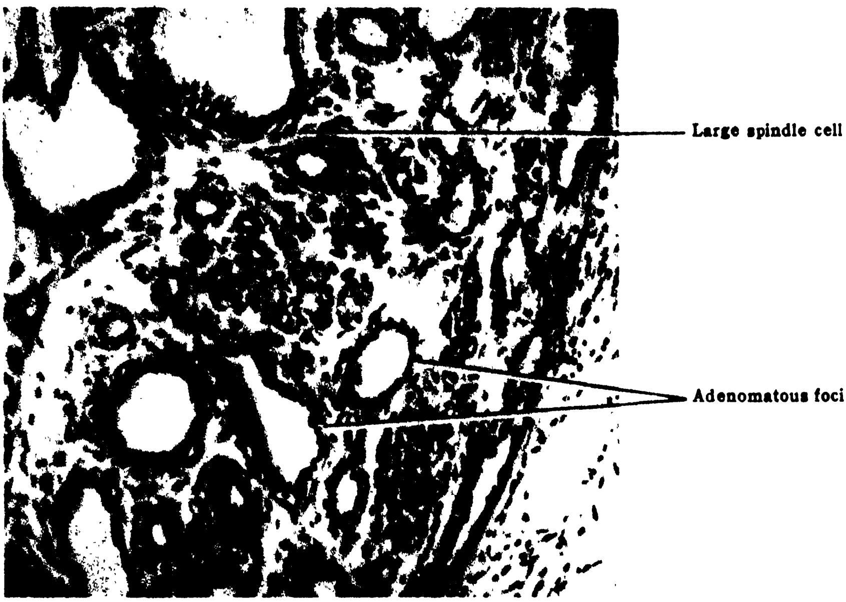


FIG. 93.—Fibro-adenoma of the mammary gland (×200).

tissue cells. It varies from the thin compact foci which barely separate the adjacent walls of many of the larger cysts to the dense compact foci around the smaller cysts. The stroma is quite cellular in the region of the capsule and blends with it. Numerous thin-walled blood vessels are scattered throughout the entire stroma.

Papillary cyst adenoma of the mammary gland.—This type is so named because of the characteristic architecture which shows large and small, branching and anastomosing irregular growths extending into cystic epithelial lined cavities (Fig. 92). These papillary structures may have one or more broad or narrow points of attachment with the remainder of the tumor. The stroma extends into these structures and thus makes up a considerable part of the papillary formations. In the tumor in general some of the

glands are nearly the same size as normal mammary gland. Most of them form irregularly shaped cysts, which vary greatly in extent and which derive their outlines from the size, shape and number of papillary growths which extend into them.

Fibro-adenoma.—This tumor of the mammary gland usually has the same type of gland-like arrangement seen in the simple adenoma. Histo-

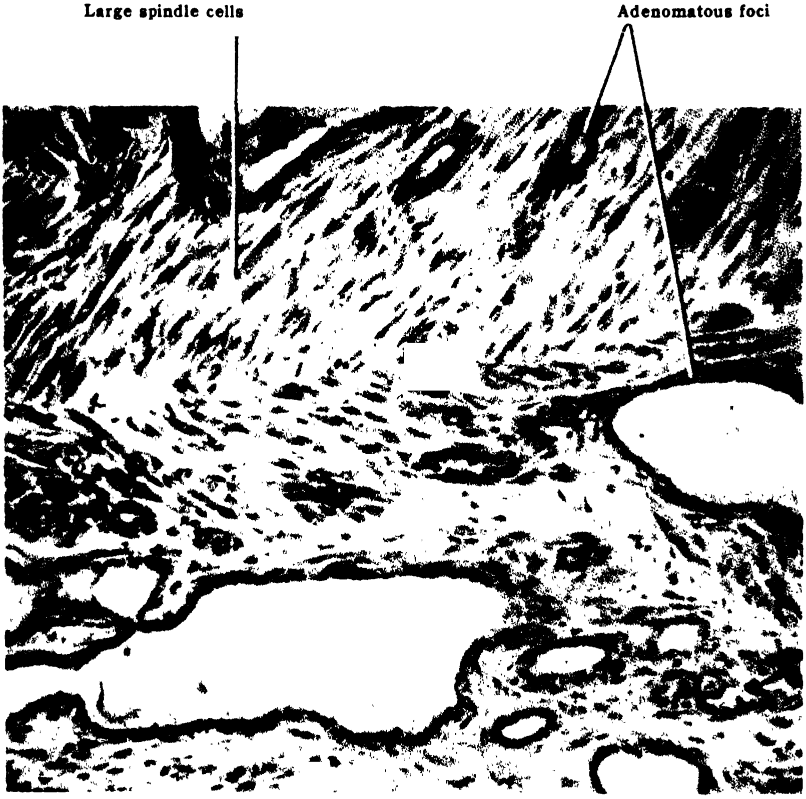


FIG. 94.—Adenofibroma of the mammary gland (×200).

logically, the stroma has the same type of wavy, thread-like connective tissue cells with spindle-shaped nuclei around the adenomatous foci. The difference between these adenomas lies for the most part in the two chief characteristics of the fibro-adenoma (Fig. 93). First, that the stroma is more abundant and makes up nearly as much of the bulk of the tumor as does the adenomatous parts. Second, that there are strands and bundles of large, closely packed connective tissue cells running in all directions throughout the stroma. These large cells are narrow, long, tapering at the ends and have centrally placed nuclei. The cytoplasm is abundant and uniform, taking a

fairly deep eosinophilic stain. Nuclei are elongated, narrow and blunt at their ends with moderately pale, coarse and fine chromatin granules.

These interlacing strands of large connective tissue cells vary in amount in different fibro-adenomas, but they are always present. When they definitely predominate over glandular parts, the tumor would then be called an *adenofibroma* (Fig. 94).

The capsule varies in thickness but not in direct relationship to the extent of their fibrous parts. Mitoses are rare throughout the entire tumor.

Adenocarcinomas of the Mammary Glands

These tumors form a group of malignant neoplasms in which stromal variations play a somewhat miner role in regard to diagnosis. However, the mammary gland epithelium gives rise to epithelial tumor cells which may assume a wide range of variations in arrangement and distribution within the stroma without becoming so undifferentiated as to lose all trace of gland origin.

A high percentage of all the spontaneous tumors which have occurred in the mice raised in our laboratory have been of mammary gland origin. Most of these mammary gland tumors have been some form of adenocarcinoma. The histological examination of these adenocarcinomas has shown that some arose from pre-existing adenomas, and some appeared to have developed directly from the mammary glands in the absence of adenomas. When a large series of these mammary tumors is examined, a few characteristic types emerge, each of which shows some variations and together they cover the various forms of adenocarcinomas observed. The tumors are classified according to the most outstanding cell arrangement. For example, a papillary cyst adenocarcinoma may have a small focus of tumor cells arranged as in intracanalicular adenocarcinoma or as in macroglandular adenocarcinoma.

Simple Adenocarcinoma.—This growth is composed of small, narrow coiled ducts which are generally evenly distributed throughout the stroma. These ducts are uniform in diameter, are about the size of the ducts of the resting mammary gland and are lined by one to two layers of cuboidal epithelial cells (Fig. 95). These cells are small, closely packed, possess a scant amount of eosinophilic cytoplasm and oval, rather hyperchromatic, nuclei.

The duct-like structures are usually so closely packed that there is little stroma between them, yet they may be spread through foci of loose stroma. The tubules are generally so coiled that the majority of them are cut in cross

section or near this angle. Mitoses are frequent and infiltration around and into normal adjacent structures, such as muscle and nerves, can be seen. Small central islands or scattered peripheral foci of other forms of mammary carcinoma are often seen in this type of tumor. The outstanding characteristics are the uniformity in size and distribution of these small duct-like, coiled structures lined by one or two layers of small, cuboidal epithelial tumor cells.

Adenocarcinoma (variable type).—This shows gland-like formations which may exhibit a wide range of size, arrangement and degree of similarity

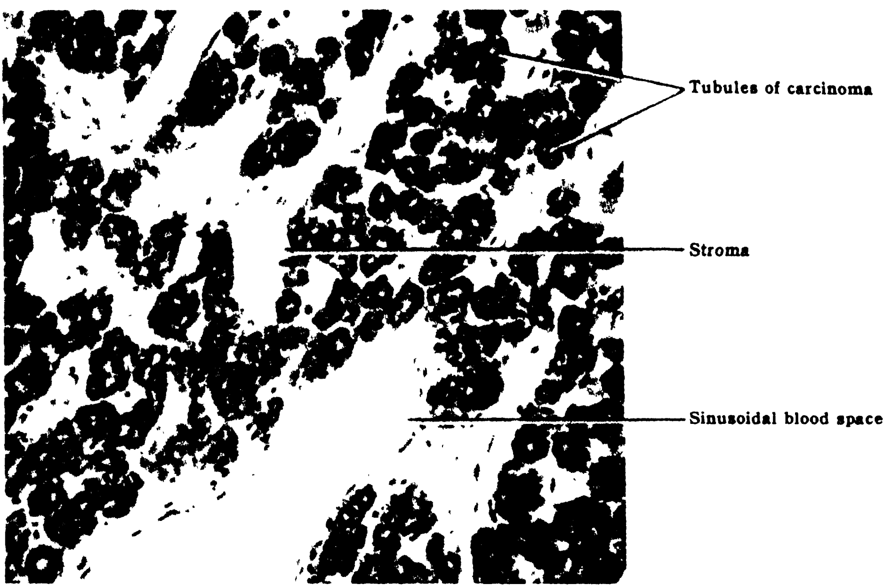


FIG. 95.—Simple adenocarcinoma of the mammary gland showing small duct-like cancer tubules (×200). The area illustrated shows more stroma than is usually observed.

as compared to the normal mammary gland. However, their origin from mammary glands is always evident, since some degree of attempted gland formation is a characteristic feature (Figs. 96 and 97). There is a varying degree of definite lumen formation, and around this the epithelial tumor cell lining ranges from one to several layers in thickness. These cells show frequent mitoses, may be large or small, cuboidal shaped and exhibit considerable loss of normal orientation. They often grade over from glands with definite lumen formations to disorganized nests of epithelial tumor cells.

Different tumors of this type may show a variety of arrangements. One may show broad, ramifying and branching strands of closely packed abortive

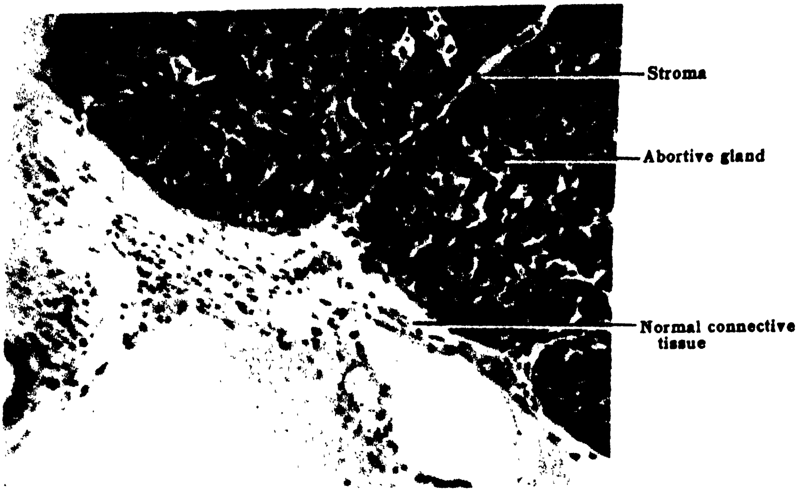


FIG. 96.—Adenocarcinoma (variable type) of the mammary gland (×200)

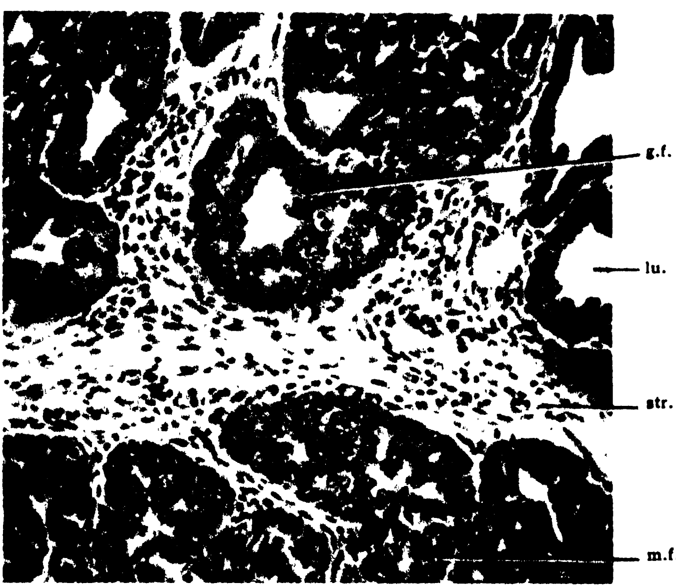


FIG. 97.—Adenocarcinoma (variable type) of the mammary gland (×200). This shows a greater gland forming tendency than is seen in Fig. 96. There is also more abundant stroma and greater mitotic activity. g.f., gland-like formations; lu., lumen; m.f., mitotic figure; str., stroma.

gland-like formations separated by thin septa of stroma with fairly large blood spaces. Another may contain large irregularly shaped nests of closely packed, poorly formed glands varying in size and lined by large and small cuboidal epithelial tumor cells with numerous, small thin-walled blood vessels and little stroma within the tumor nests but with dense stroma separating them. A third type may exhibit pseudoglandular arrangements of large and small, or fairly uniform size, imperfectly formed glands about a focus or stroma which consists largely of a thin walled blood vessel, or about a necrotic focus of tumor cells. Some are composed of clusters of large and small, blood-filled endothelial lined spaces, surrounded by poorly formed glands which may be markedly compressed by the blood spaces. Still other types are seen where there may be metaplasia producing true epithelial pearl formations with the stroma varying in amount and density. This description does not cover completely all the varieties which might be observed for this tumor type.

The distinguishing characteristics are some degree of attempted gland formation by the majority of the epithelial tumor cells. The glands may vary in size and arrangement and are lined by large or small cuboidal epithelial cells. Gland walls vary from one to several layers in thickness and show frequent loss of normal orientation of the cells where the walls have become thickened. Mitoses are abundant. Infiltration of the surrounding tissues and metastases to the lungs are often observed.

Papillary cyst adenocarcinoma.—At least a large proportion of this group arises in pre-existing papillary cyst adenomas. For this reason there is a striking similarity in the general arrangement of the stroma in both the benign and malignant tumors. However, in the latter the stroma is frequently less abundant, except at the base of and within the central portion of the papillary structure. The epithelial tumor cells cover the surfaces of the poorly defined cysts and the branching papillae. On the latter they often form irregular finger-like projections which contain a small amount of connective tissue extending from the central stromal core (Fig. 98). In the larger papillae the distal portions are composed chiefly of epithelial tumor cells. These cells may be arranged in groups of gland-like formation, nodules, sheets of cells or a combination of these with or without imperfectly formed glands of different sizes. Even in the larger tumor masses thin strands of stroma can be found in the form of scattered groups of small connective tissue cells and small, thin-walled blood vessels.

The epithelial tumor cells are medium sized, cuboidal or low columnar, with oval, moderately hyperchromatic nuclei containing scattered chromatin

granules. The cytoplasm is fairly abundant and eosinophilic. These cells vary somewhat in size. Mitoses are fairly frequent. On the surfaces of the papillae and within the gland-like formations the epithelial cells vary from one to several cell layers in thickness and normal orientation is frequently lost. Invasion of surrounding normal structures and metastases to the lungs occur.

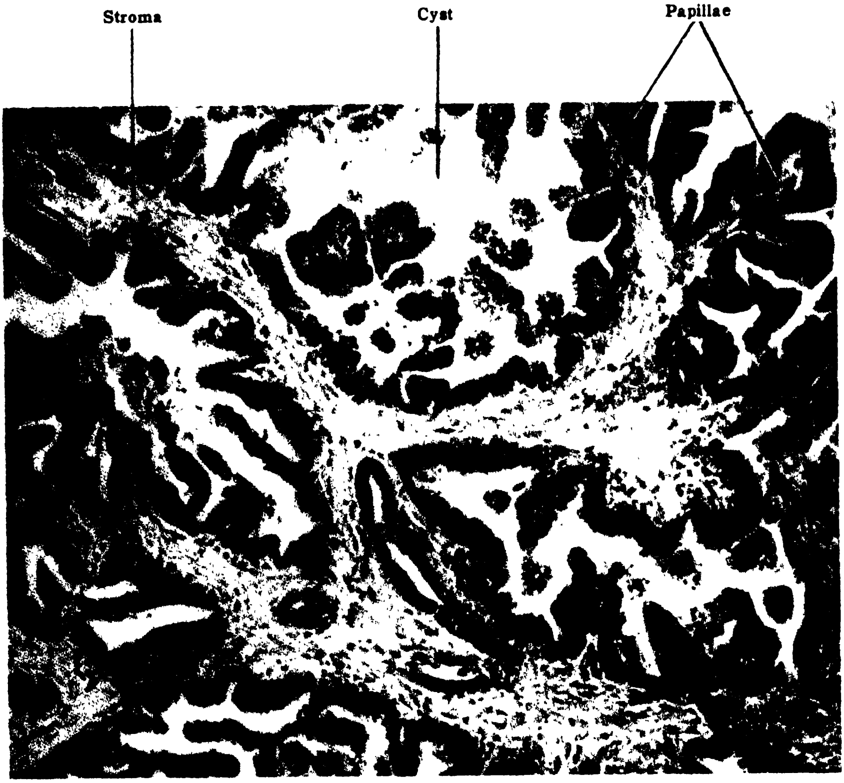


FIG. 98.—Papillary cyst adenocarcinoma of the mammary gland (×200).

The distinguishing characteristics are large and small branching and anastomosing papillary tumor growths within cyst-like cavities which are often so filled with these papillary structures that the cysts are poorly defined. The walls of the cysts are lined by medium sized cuboidal or low columnar epithelial cells which also extend over the surfaces of the branching papillary formations. Here they form a cover of one to several cell layers in thickness. The stroma forms a definite core of connective tissue containing thin-walled blood vessels in the papillary structures, and the stroma may not be clearly defined in the distal portions of their branches. However, stromal

connective tissue and blood vessels infiltrate and can be found, by careful observation, even within the finer branches of the papillae which are composed largely of disorganized nests and partial gland-forming foci of epithelial tumor cells.

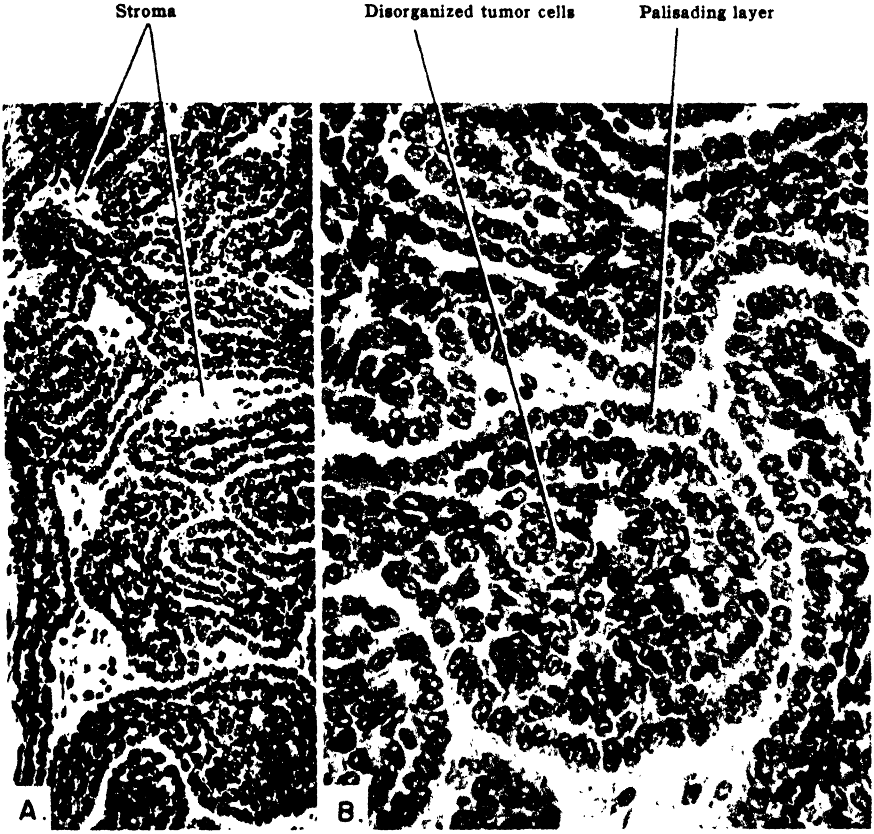


FIG. 99.—Intracanalicular adenocarcinoma of the mammary gland. A, shows typical architecture (×200); B, shows the cellular detail (×400).

Intracanalicular adenocarcinoma.—This tumor grows as finger-like branching and anastomosing strands of epithelial tumor cells extending into a loose stroma (Fig. 99A). The edges of these strands are smooth due to an orderly palisade arrangement of a single outer layer of epithelial cells. Within this palisade layer the entire remainder of these finger-like processes is composed of epithelial tumor cells of the same type and size but with a disorderly arrangement due to loss of normal orientation (Fig. 99B).

These tumor cells are closely packed and cuboidal in shape, somewhat larger than normal resting mammary gland epithelium, with moderately

hyperchromatic, oval nuclei and scant cytoplasm. The stroma consists of loosely scattered, threadlike connective tissue cells and thin-walled blood vessels.

This type of tumor derives its name from its manner of growth, which is intraductal, filling the lumen with wildly growing, epithelial tumor cells, but having an outer layer of orderly arranged cells. This arrangement of cells, together with the manner of infiltrating the stroma in branching finger-like



FIG. 100.—Macroglandular adenocarcinoma of the mammary gland (×200).

processes, constitutes the distinguishing characteristics for intracanalicular adenocarcinoma.

Mitoses are frequent. Infiltration of normal adjacent structures occurs and metastases to the lungs are fairly frequent.

Macroglandular adenocarcinoma.—This type of growth is probably what Apolant (1) called fissure forming carcinoma of the mammary gland. Here occurs what appears to be enormous gland-like structures with long, branching, irregular lumena (Fig. 100). The walls are thrown into folds and are composed of four or five to many cell layers in thickness. The epithelial tumor cells forming the walls are medium sized, oval and closely packed, often growing in wild disorder with frequent mitotic figures in evidence.

They have a small amount of eosinophilic cytoplasm and oval, somewhat hyperchromatic nuclei.

The lumena of these glands are very prominent. Between the glands the supporting stroma may be reduced to narrow but conspicuous septa of dense fibrous connective tissue containing some thin-walled blood vessels.

The chief characteristic of this type of tumor is the enormous, irregularly branching, duct-like structures whose lumena may extend for considerable distances. The walls, which are composed of compact tumor cells, are from four or five to twenty or more cells in thickness and follow fairly closely the contours of the lumena. This gives the appearance of giant thick-walled ducts. Invasion of adjacent normal tissue is commonly seen and metastases in the lungs are often found.

In quite a number of breast tumors there can be seen large and small, blood-filled, cyst-like spaces which are often clustered closely together. These are always surrounded by small epithelial tumor cells, which may form compact strands varying from three or four to many cell layers in thickness. These cells may or may not show some flattening where they come in close contact with the blood-filled cyst-like spaces. These spaces have an acellular, membrane-like, eosinophilic zone between the epithelial cells and the blood. In some instances there are scattered, flattened cells present which suggest an endothelial lining within these spaces.

Some investigators have considered these cystic tumors as belonging to a type called *hemorrhagic cyst adenocarcinoma* (Fig. 101B). One can find simple adenocarcinoma with foci where clusters of blood filled spaces are separated by thin, compressed strands of epithelial tumor cells. Similar spaces are also frequently found in cases of adenocarcinoma, variable type, as well as in nests of tumor cells which are in the midst of and continuous with intracanalicular adenocarcinoma and even papillary cyst adenocarcinoma. In the latter type papillary growths may extend into the cyst-like blood filled spaces. When this is taken into consideration, it may be advisable to consider these hemorrhagic cysts not as a separate type but more as a common characteristic of adenocarcinomas in general.

In gross observation these tumors show many bulging, blood filled cysts. The tumor is turgid and when cut open will collapse into a soft hemorrhagic mass.

There is a somewhat similar situation in the case of the frequent appearance of epithelial pearls, composed of cornified, squamous epithelial cells grouped in concentrically arranged foci (23). These pearls can be found in all types of adenocarcinoma. This may even progress to the stage where the

tumor shows a central amorphous mass which grades over into desquamated stratified squamous epithelium. This in turn grades over into definite adenocarcinoma. This can be considered as *adeno-acanthoma*, but a more probable explanation is that metaplasia has occurred changing glandular epithelial cells into stratified squamous epithelium (Fig. 101A).

These two characteristics in their most extreme manifestations could be considered as special types of breast cancer. However, an interpretation of



FIG. 101.—Two features frequently observed in adenocarcinoma of the mammary gland. A, metaplasia of the glandular epithelium in a nest of tumor cells (center of figure) to keratinized squamous epithelium (×200); B, cystic blood-filled spaces surrounded by the tumor cells of adenocarcinoma (×200).

the histological picture presented by the various forms assumed by adenocarcinoma of the mammary gland probably does not require these subdivisions.

Carcinoma Simplex of the Mammary Glands

Histologically this tumor is so undifferentiated that its appearance is frequently difficult to associate with that of the mammary gland from which it originated (Fig. 102). However, one can find small foci and traces of adenocarcinoma that blend with the carcinoma simplex cells forming the bulk of the tumor mass.

The architecture of the tumor shows compact masses of epithelial tumor cells growing in long, broad, branching and anastomosing bands or in a com-

pact mass without any definite arrangement and with rather inconspicuous stroma. In the first type there is often considerable debris resembling necrotic material between the bands of tumor cells, and pseudoglandular arrangement around this debris and surrounding the thin-walled blood vessels is not uncommon. Clusters of large and small, blood filled, cystic spaces similar to those observed in adenocarcinoma are sometimes found in this type of tumor.

The epithelial tumor cells are usually quite large and vary in size. They are compactly arranged with rather indistinct cell boundaries. In outline

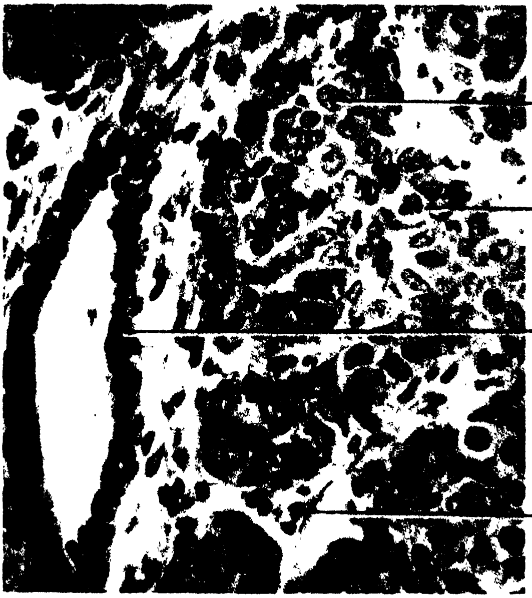


FIG. 102.—Carcinoma simplex of the mammary gland (×400).

the cells vary from rounded and polyhedral to somewhat spindle-shaped. Their nuclei are hypochromatic, have one to many nucleoli, are round to nearly spindle-shaped, and vary in size. Mitoses are frequent. Some mononuclear tumor giant cells are present. The cytoplasm is pale, eosinophilic, and varies from a scant amount in the rounded cells to abundant in the spindle-shaped epithelial tumor cells and in the polyhedral cells. The stroma is usually represented by numerous, large and small, thin-walled blood vessels with a small amount of connective tissue, except between large nests of tumor cells where well defined septa of stromal connective tissue are present.

The rounded and spindle-shaped epithelial tumor cells can often be found in the same high power fields. The latter can be seen arising from the

rounded epithelial tumor cells and represent a more undifferentiated form of carcinoma simplex. These spindle cells often grow in nests and strands with dense irregular strips of connective tissue cells scattered between them. In some respects they may be confused with fibrosarcoma. However, fibrosarcomas have more distinct cell boundaries and the cells are more definitely tapering and spindle shaped. Also, these cells usually have less cytoplasm and possess smaller, more hyperchromatic, nuclei which are more pointed at the poles. The spindle-shaped carcinoma cells are greatly elongated epithelial cells and grade into polyhedral and rounded epithelial tumor cells at the periphery of the tumor cell nests.

The polyhedral cells are the most uncommon carcinoma simplex cell in our stocks. The cases we have show pale, rather large, many sided cells which in some respects resemble squamous cells of the epidermis, but are without keratinization. They grow in closely packed, irregular masses with dense stroma between them. Their origin can be traced to mammary gland epithelium. Mitoses are frequent and the tumor freely invades surrounding tissues.

Carcinoma simplex as a whole grows rapidly, shows extensive infiltration into adjacent tissues and metastasizes to the lungs. Epithelial pearls may be found, especially in the branching and anastomosing forms.

Carcinosarcoma of the Mammary Glands

This type originates from a pre-existing fibroadenoma. In this tumor the adenomatous elements become malignant as shown by invasion of the basement membrane, followed by the spreading of the epithelial tumor cells into the stroma in dense, irregularly arranged nests. There is also a malignant change involving the large connective tissue cells found in the stroma. These multiply and spread as interlacing strands of connective tissue tumor cells. Thus the resultant histological picture is that of a fibrosarcoma growing around nests of adenocarcinoma (Fig. 103). Both types of tumor show frequent mitoses. Should the sarcoma outgrow the carcinoma, the picture is predominantly that of fibrosarcoma.

Fibrosarcoma of the Mammary Gland Stroma

This may originate from a carcinosarcoma, as above, or from an adenofibroma in which the fibromatous elements alone have become active. This tumor can also originate from the stroma about the mammary glands in the absence of an adenoma. The resultant fibrosarcoma forms a dense tumor mass composed of closely packed spindle-shaped connective tissue tumor

cells. The architecture exhibits the same characteristics as are described under fibrosarcomas of the subcutaneous tissue in general. Mammary glands are present around this tumor and usually are invaded by the infiltrating tumor mass.

Tumors in or Near the Mammary Line and Its Branches but Not Originating from the Mammary Gland Proper

Tumors in or near the mammary line and its branches and not originating from mammary glands or their stroma may be either benign or malignant. While such tumors are in no essential way different from tumors in general

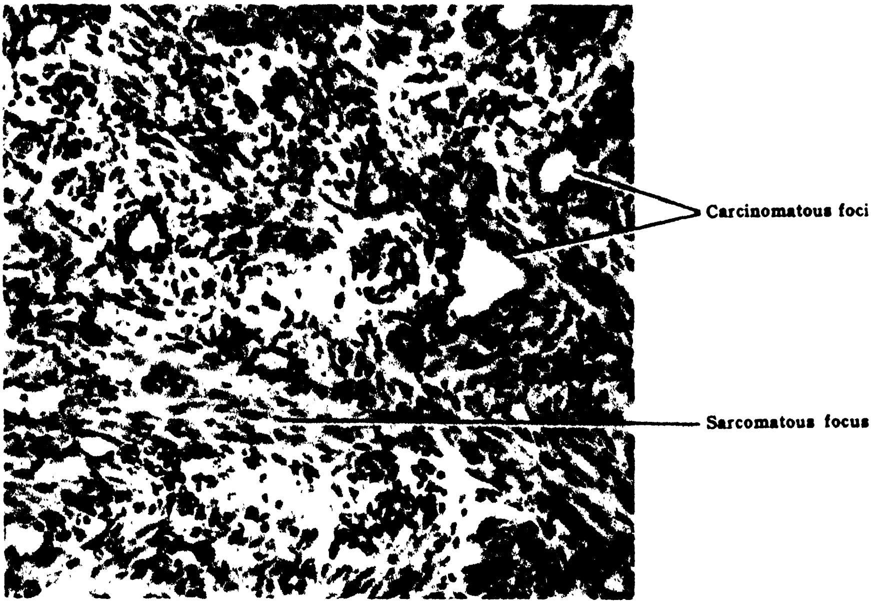


Fig. 103.—Carcinosarcoma of the mammary gland (×200).

which are found subcutaneously, it is convenient to list and briefly describe them here because of the fact that confusion with true mammary tumors is possible if diagnosis is not carefully made. Normal appearing mammary glands are found either adjacent to or invaded by these tumors. For the details of their histological appearance the reader should turn to the section on Tumors of the Dermis, Subcutaneous and Body Wall Tissues (p. 199). Only the names and a brief description are given below.

Fibromas, chondromas and osteomas.—These originate from fibrous connective tissue, cartilage and bone respectively and are uncommon in this

region. At least this is true for our mice and according to the published literature from other sources.

Angiomas.—These tumors are found especially in the C57 black stock mice. This is true for both *hemangiomas* and *lymphangiomas*. The former has been mistaken for primary carcinoma of the mammary gland on superficial inspection of the living mouse. Even on gross section it may resemble somewhat the mammary gland tumors with dilated, blood-filled cysts.

Hemangiomas may be formed anywhere in the mammary gland region. They are composed of the elements of blood vessel walls and develop as a benign tumor with a poorly formed capsule. *Lymphangiomas*, on the other hand, are found in the axillary or the inguinal regions. They are benign tumors formed from the elements of the lymph vascular system.

Lipomas.—These are tumors of adipose tissue. They are benign tumors with cells larger than normal and without the vascular arrangement of normal fat tissue.

Fibrosarcoma.—This growth may occur near the mammary glands and may invade and destroy them. Here the epithelium is not a part of the tumor. The tumor has spread around the normal tissue as is the case in the infiltration of other normal tissues. Fibrosarcoma here is the same as that which will be described later under subcutaneous fibrosarcoma (Fig. 112).

Melanoma.—This is a pigmented tumor sometimes seen at the base of the tail in the dba females which are of a high mammary tumor stock. However, the tumor is usually black and not confused grossly with a mammary carcinoma, even when the latter has blood filled cysts.

Rhabdomyosarcoma.—This is a sarcoma of the striated muscles and has been found in the mammary gland region of dba stock female mice. The same is true of hybrids between the dba and C57 black stocks.

Osteogenic sarcomas and chondrosarcomas.—Sarcomas of bone origin in the mammary region are uncommon in our stocks. One chondrosarcoma originating from rib cartilage has been noted. As stated under the subcutaneous tumors, osteogenic sarcoma has occurred several times. J. A. Murray (43) reported or a chondro-osteosarcoma in the left groin of a female mouse. Pybus and Miller (45) developed a branch of the Simpson strain with a fairly high incidence of bone sarcomas, several of which were in the mammary region.

Carcinoma of skin appendage.—These arise from specialized sebaceous glands. The preputial (23) and clitoral glands also fall into this group. This carcinoma can be traced to the skin appendage glands as its site of origin (Fig. 109).

TUMORS OF THE SKIN, SUBCUTANEOUS AND BODY WALL TISSUES

Tumors of the dermis, subcutaneous tissues and body wall may be considered together. A tumor of the epidermis is fairly easy to determine grossly, but tumors of the dermis might be confused with many of the new growths occurring in the subcutaneous and body wall tissues. In the mammary line and its branches the tumors not of mammary gland origin would be the same as those of the subcutaneous tissues in general, except for those of the axillary and inguinal lymph nodes. The majority of these lymph node tumors belong in the groups to be discussed under lymphocytomas, myelocytomas and monocytomas.

TUMORS OF THE EPIDERMIS

Tumors of the epidermis are not common in any of our stocks. Papillomas and epidermoid carcinomas have been found in small numbers in many of the stocks, chiefly in the C57 black, X, W, ce, dba and their hybrids. Papillomas occur most frequently on the external genitalia of the female, around the anus, on the eyelids, ears, lower lip and occasionally on the skin of other parts of the body. Epithelial horns are rare but have been found about the head and shoulders in the C57 black and the dba mice. Epidermoid carcinomas have been seen arising from the skin of the dorsal and ventral surfaces (Fig. 106), the shoulders (Fig. 107), the lower lip (Fig. 105), the eyelid and the skin around the anus and external genitalia of the female. Frequently the epidermoid carcinoma occurs within a preexisting papilloma.

The Papillomas.—These are benign epithelial tumors which are elevated above the skin surface, often pedunculated, and contain varying amounts of stroma. The epithelium is the active part of these tumors and shows thickening and overgrowth. This results in the formation of the blunt, elevated papillae and in the varying degrees of epithelial downgrowth into the dermis (Fig. 104). Within the thickened epithelial layer the normal orientation of epidermis is not lost and the basement membrane is intact, but marked keratinization and cornification are usually present. The elevated mass may consist almost entirely of epithelium with only thin finger-like processes of stroma extending between the irregular epithelial downgrowths and forming a central core within the papillae.

Epithelial horns are really papillomas which show a marked degree of the piling up of the cornified epithelium until grossly a horn-like growth about two centimeters long may develop. This structure tapers from the base to

the tip. At the base the epithelial downgrowths may penetrate below the level of the epidermis and contain only thin, loose strands of stroma (Fig. 104). The basement membrane remains intact and the arrangement of the epithelium at the base is that of a papilloma.

Epidermoid carcinomas of the skin.—These are malignant tumors of the epidermal epithelium. They vary from early forms beginning in papillomas, such as on the lip (Fig. 105A) or external genitalia, to wildly growing types

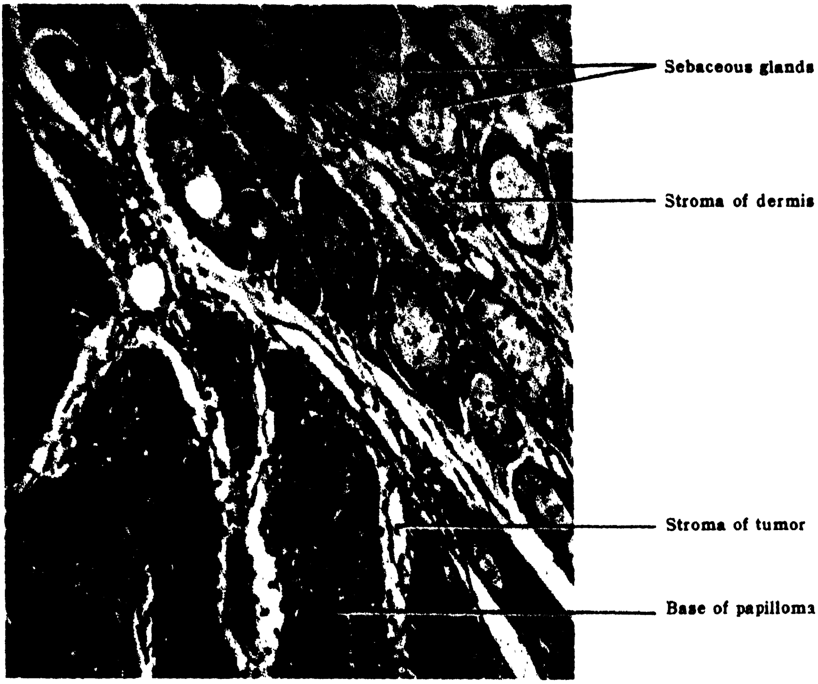


FIG. 104.—Subcutaneous focus from the base of an epithelial horn (papilloma) of the skin (×200).

with marked anaplasia. This is accompanied by extensive invasion and occasional metastases to lymph nodes (Fig. 108A) and lungs. The low grade forms show loss of orientation, extension through the basement membrane and invasion of the adjacent normal structures. Except in the most rapidly growing forms, marked keratinization and extensive formation of large and small epithelial pearls are common (Fig. 106). The epithelial tumor cells grow in nearly solid masses without much stroma. Epithelial pearls are usually scattered throughout and show concentrically arranged, flattened, cornified epithelial cell debris that takes an eosinophilic stain. Around these pearls are irregular clumps of large polyhedral cells with large,

pale, oval frequently pyknotic, nuclei. The cytoplasm is abundant, acidophilic and often contains coarse keratohyalin granules. These cells grade over into smaller, closely packed, disorderly, polyhedral to somewhat spindle-shaped cells. They possess a relatively small amount of eosinophilic cytoplasm and contain oval, moderately hypochromatic nuclei with scattered coarse chromatin granules. Scattered foci of brown pigment resembling melanin are often seen. Mitoses are frequent in these smaller cells.

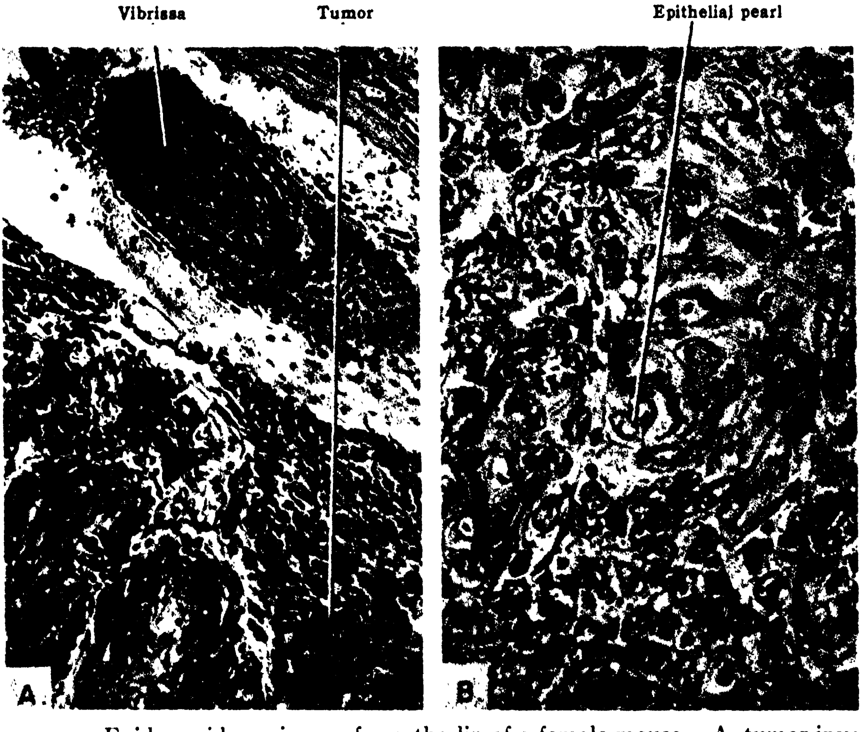


Fig. 105.—Epidermoid carcinoma from the lip of a female mouse. A, tumor invading dermis around vibrissa (×200); B, cellular detail of this same tumor (×400).

Occasionally a very malignant form shows little epithelial pearl formation (Fig. 107). It may consist of wildly growing spindle-shaped epithelial tumor cells that blend with narrow strands and small nests of the smaller types of polyhedral epithelial tumor cells (Fig. 108B). Unless the origin can be traced to the epidermis in this type of tumor, the architecture is so misleading that it could confuse one in interpreting the histopathology. Mitoses are abundant.

Carcinomas of skin appendages.—These all give the same general picture. They originate in the specialized sebaceous glands of the head region of

males and females, most commonly in the A stock. They are also seen arising from the preputial glands of the male (23) and the clitoral glands of the female mouse. The chief characteristic is the resemblance of the cells to the normal cells of the sebaceous glands. They are large round cells with pale cytoplasm which appears to be filled with fine droplets (Fig. 109). The nuclei are relatively small, pale, oval and centrally located. These tumor cells grow in irregular masses as well as in broad branching strands. The

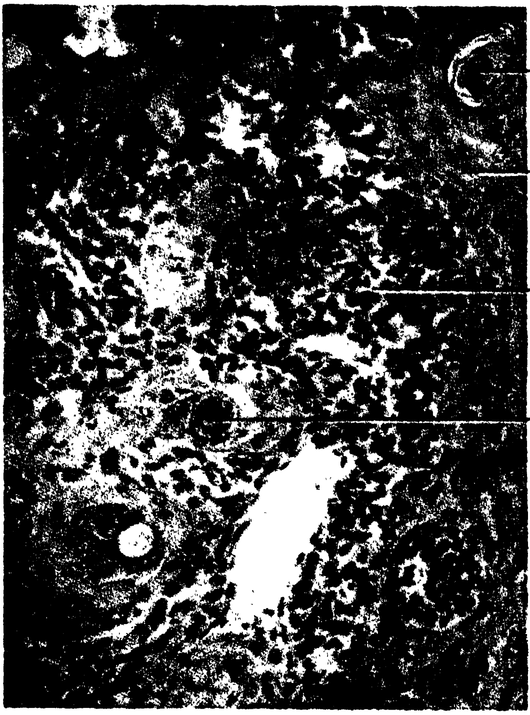


FIG. 106.—Epidermoid carcinoma of the skin on the ventral abdominal surface (×200).

most rapidly growing parts may contain small round, rather deeply staining, cells which have a small amount of cytoplasm and oval nuclei. These cells resemble the small undifferentiated cells of epidermoid carcinomas. Sometimes stratified squamous cells are found in some of the tumors of the clitoral glands. Since the smaller tumor cells may also rather closely resemble small undifferentiated cells of carcinoma of the mammary gland, it is not always easy to determine whether one is looking at a carcinoma of the clitoris or a carcinoma of the mammary gland invading the clitoral glands. Both of these conditions do occur. Usually the clitoral glands show active growth with dedifferentiation when they are the primary site of the neoplasm.

TUMORS OF THE DERMIS, SUBCUTANEOUS AND BODY WALL TISSUES

Many tumors of the dermis and subcutaneous tissues are not easily separated, and for the purposes of this section no attempt to separate them will be made. Benign and malignant forms are found here. These are representative of the type of tissues normally found in the subcutaneous and body wall region.

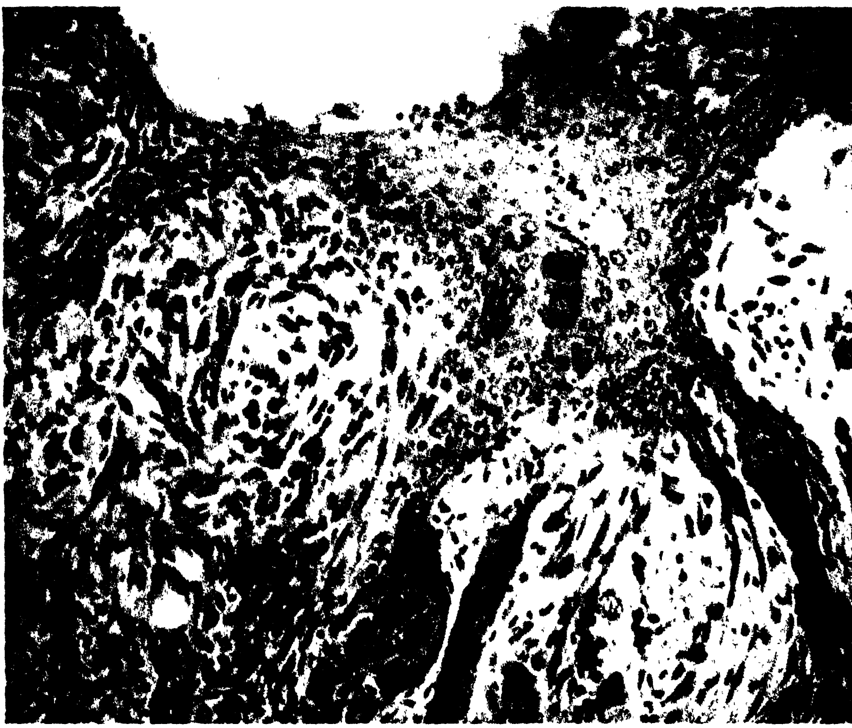


FIG. 107.—Rapidly growing epidermoid carcinoma of the skin (×200). This shows little epithelial pearl formation in contrast to Fig. 106.

Fibroma.—This is a benign tumor, not commonly observed, composed of connective tissue cells. These cells are uniform in size and shape and are distributed throughout the intercellular substance. The tumors are encapsulated and invasion does not occur. Mitotic figures are rare.

Chondroma.—This is a benign tumor originating from cartilage. The cartilage cells are atypical, larger than normal and arranged in irregular islands. They show a tendency to mucoid degeneration or calcification. Blood vessels may be fairly abundant. Mitoses are rare and a well formed capsule is present. This type of tumor is not common among our stocks.

Osteoma.—This growth originates from bone and is a benign encapsulated tumor. It is composed mostly of dense compact bone, usually with little marrow (Fig. 110). This is another uncommon form of tumor and is probably overlooked when small and inconspicuous.

Lipoma.—This is a benign tumor of fat or adipose tissue and is usually composed of large fat cells. The tissue looks nearly normal but lacks trabeculae, normal vascularity, and the fat cells are larger than ordinary. Due to the lack of normal vascularity retrograde changes may occur, such as necrosis followed by calcification. This tumor has been seen in the yellow stock, in which there is a tendency for the mice to become obese.

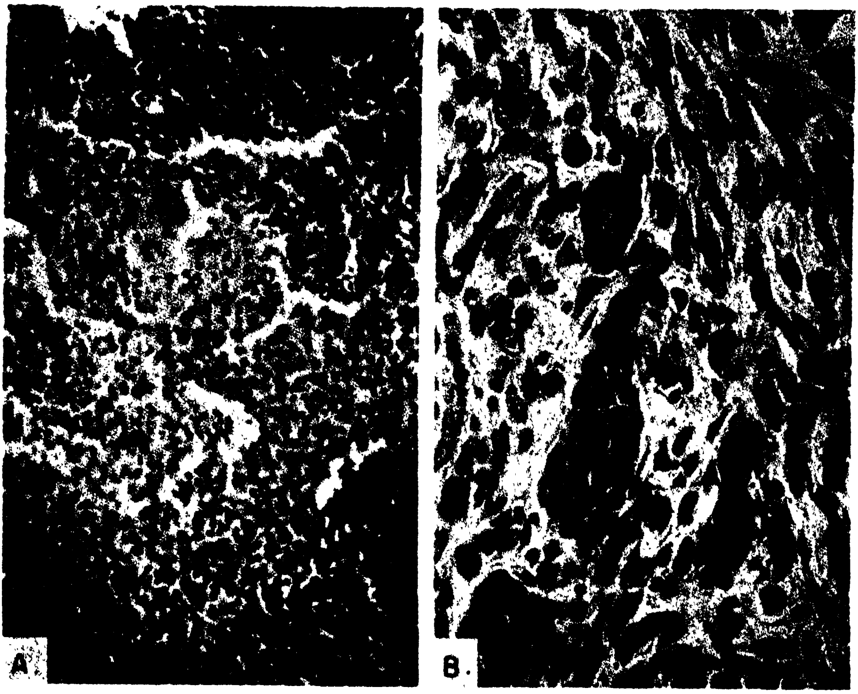


FIG. 108.—Epidermoid carcinoma of the skin. A, inguinal lymph node metastasis of the carcinoma shown in Fig. 106 (×200); B, cellular detail of the tumor in Fig. 107 (×400).

Angiomas.—These are benign and are composed of the elements of either the blood vascular or the lymph circulatory systems. When they are formed from blood vessels they are called hemangiomas, and lymphangiomas when formed from lymph vessels.

Hemangiomas grow either as a diffuse mass or as closely clustered groups of endothelial lined spaces supported by a dense stroma. The endothelial



FIG. 109.—Carcinoma of skin appendage origin from the head region (×200).



FIG. 110.—Osteoma of a tail vertebra (×75).

cells are flattened and pavement like. The spaces are blood filled and irregular in size and shape (Fig. 111A and B). Only narrow septa of fibrous connective tissue stroma are present between the spaces. Epithelial elements do not appear as a part of these tumors. Within the tumors foci of thrombosis and considerable old blood pigment are often seen. The connective tissue capsule is not well formed. Mitotic figures are rare. In mice this tumor

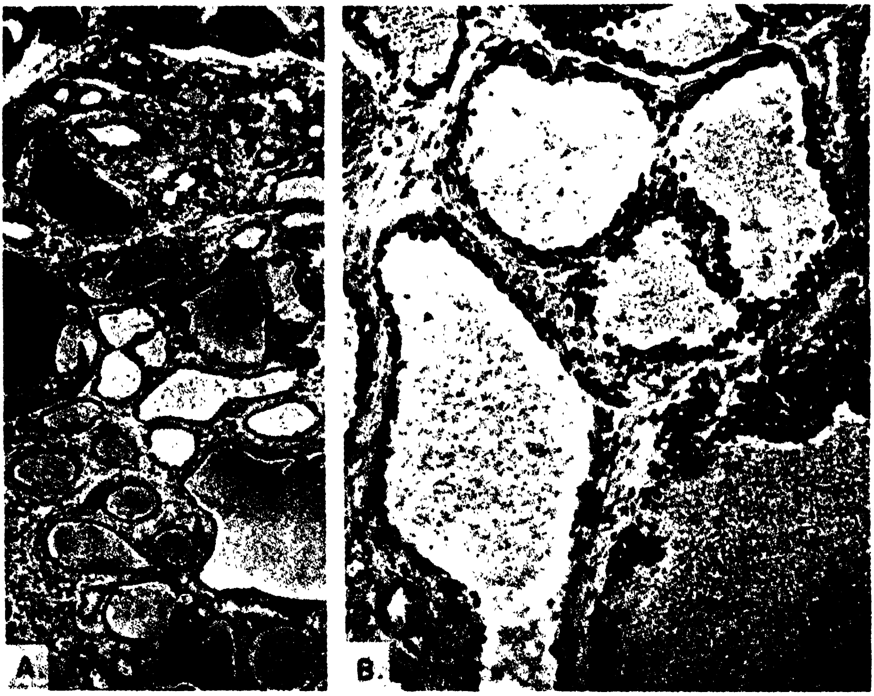


FIG. 111.—Hemangioma. A, shows typical architecture (×50); B, shows blood-filled endothelial lined spaces (×200).

frequently shows a mixture of capillary-like and cavernous blood filled spaces.

Lymphangioma is most often found in the axillary and the inguinal regions. Sometimes it occurs near these sites where it may have originated from lymph nodes. Irregular, large or small lymph filled sinusoidal spaces are seen lined by flattened endothelial cells. The connective tissue stroma forms nodular septa containing small lymph vessels and normal appearing lymphocytes in varying degrees of concentration. In its most benign form this tumor exhibits broad bands of connective tissue stroma surrounding long, narrow, irregular, endothelial-lined spaces filled with lymphocytes. This may involve a large part of a lymph node.

Fibrosarcoma.—Among the more common malignant tumors in the subcutaneous region, the fibrosarcoma is the tumor most often observed. However, no stock of mice shows a frequency of subcutaneous tumors which would enable one to call it a high tumor stock in that respect. It is unusual to find a stock showing over 15 per cent of the mice with tumors other than mammary carcinoma in this region. Many lines of mice show considerably less.

Fibrosarcoma originates from the fibrous connective tissue cells. The basic architecture is the same whether it develops in the mammary line and

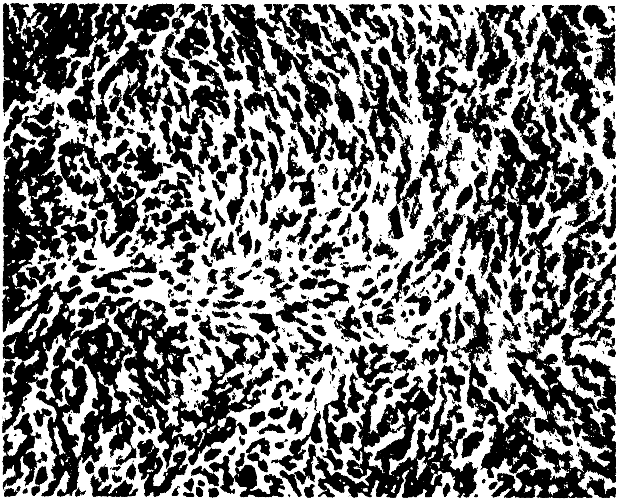


FIG. 112.—Fibrosarcoma of the subcutaneous connective tissue showing the typical interlacing pattern of the spindle cells (×200).

its branches or in any other subcutaneous focus. Grossly, the tumor is a compact mass with a smooth, rounded, white surface. The cut surface is uniform, bulging and varies from soft to firm. Microscopically it shows closely packed spindle-shaped tumor cells (Fig. 112). Their architecture presents a picture of solid masses of cells alternating with large and small whorls and interlacing bands of fibrous connective tissue tumor cells. The tumor cells exceed the stroma and the latter is difficult to identify, but appears to be represented by inconspicuous stromal connective tissue cells surrounding the individual tumor cells. Fibrosarcoma tumor cells often appear slightly separated, as though shrunken away from the intercellular stroma. Small, endothelial lined thin-walled blood vessels are abundant. Invaded tissues such as striated muscle, nerves, large blood vessels and mammary glands may be seen, for no capsule is present and infiltration occurs.

The sarcoma cells vary in size from medium to large, while very large tumor giant cells are sometimes present. In shape the tumor cells range from blunt, to long, narrow spindle cells. The cytoplasm is pale, eosinophilic and appears to have faint, longitudinal striations. Nuclei are elongated, moderately hypochromatic, more or less irregular in outline, and have one or more large nucleoli. The nuclei are centrally located and between the nucleated cells are many smaller non-nucleated fragments. These fragments represent the tapering ends of long cells cut at such an angle that the nuclei are not included. Mitotic figures are abundant.

In some undifferentiated fibrosarcomas the spindle-shaped cells are often in the minority. These tumors show many polyhedral cells that are large, pale and closely packed. They grade into very large mononucleated and multinucleated tumor giant cells. These have an irregular outline and abundant, rather deeply eosinophilic, cytoplasm. Some of the largest cells may have a stippled appearance due to the presence of tiny vacuoles. This is a degenerative change which can advance into a signet ring type of cell where the nucleus and cytoplasm are compressed into a small peripheral mass. As a rule the more undifferentiated the cells, the less the amount of stroma and the more rapid the growth of the tumor.

Liposarcomas.—These are malignant tumors originating from fat tissue as in a lipoma. They are among the rare tumors in mice, but have been observed in yellow stock animals.

Neurogenic fibrosarcoma.—This type is difficult to separate from fibrosarcoma of connective tissue origin in the mouse. However, it can be identified when the origin is definitely traced to nervous tissue. There is also a more marked tendency to show a herring bone pattern type of arrangement of the spindle-shaped tumor cells in neurogenic fibrosarcoma. It is probable that the rapidly growing undifferentiated tumors of this group are often classed with the fibrous connective tissue tumors, fibrosarcomas.

Melanomas.—These tumors have occurred for the most part in our dba stock and the common site has been on or near the tail. There have been cases of melanoma of the eye, ear and the skin in general. The tumor is grossly brown to black and the color is often visible through the skin of the living mouse. The tumor is smooth and rounded and may show tiny black foci extending into the adjacent tissues. Lymph nodes when involved often appear black to the naked eye, and lung metastases may be so extensive that the lungs are sometimes solid and nearly black in color. The cut surface is bulging, smooth and black or nearly black.

These tumors are usually malignant and the majority of them may properly be called *malignant melanomas*. There is neither space nor neces-

sity here to enter into the controversy over the exact tissue of origin and whether they should be called melanocarcinomas or melanosarcomas. For our purposes it is sufficient to designate them as either melanomas or malignant melanomas.

The histopathology usually shows a tumor whose architecture and cellular detail is heavily masked by the intense pigmentation (Fig. 113). Around the edge of the tumor the cellular detail is visible and shows large and oval or smaller and spindle-shaped cells whose cytoplasm is filled with a closely

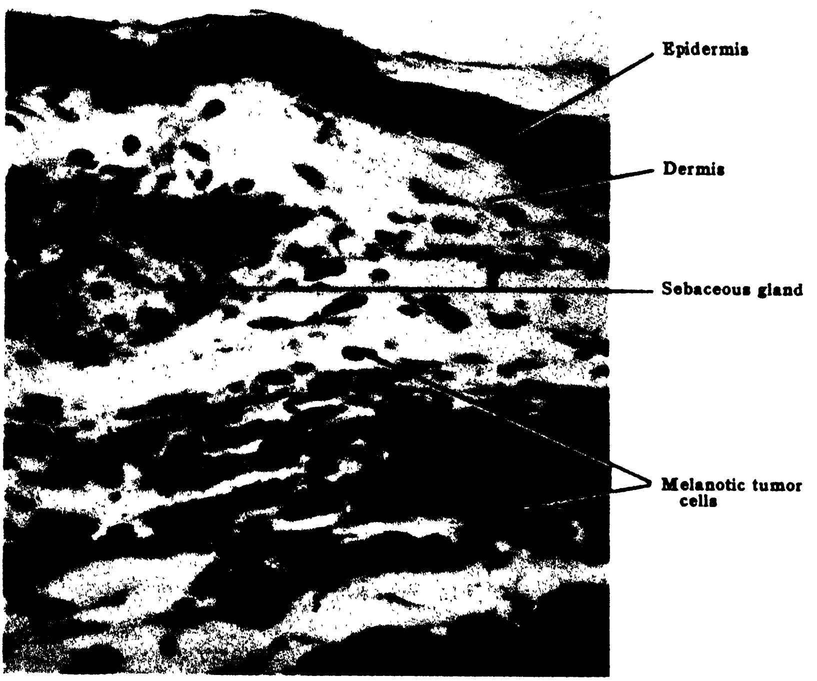


FIG. 113.—Melanoma of the skin (×200).

packed fine brown pigment, melanin. The most rapidly growing foci show the least pigmentation and the cellular detail is easily seen under the microscope. Mitotic figures are often abundant and invasion of the adjacent tissues is extensive. This is one of the most widely metastasizing types of tumors found in the mouse.

Rhabdomyosarcoma.—This is a malignant tumor originating from striated muscle (Fig. 114). In the subcutaneous region it appears to occur generally in mice of about the same age as animals bearing other types of subcutaneous tumors. However, cases are sometimes observed in young mice probably from embryonic rests in the striated muscle. The earliest

case in our records occurred in a two and one half months old dba female.

This tumor is composed of cells which resemble embryonic muscle growing in wild confusion. These can be seen to originate from normal muscle and may become spindle cells which resemble spindle cell sarcoma of fibrous connective tissue origin.

The tumor cells are eosinophilic but paler than normal muscle. The largest cells are most differentiated and possess finely granular cytoplasm.

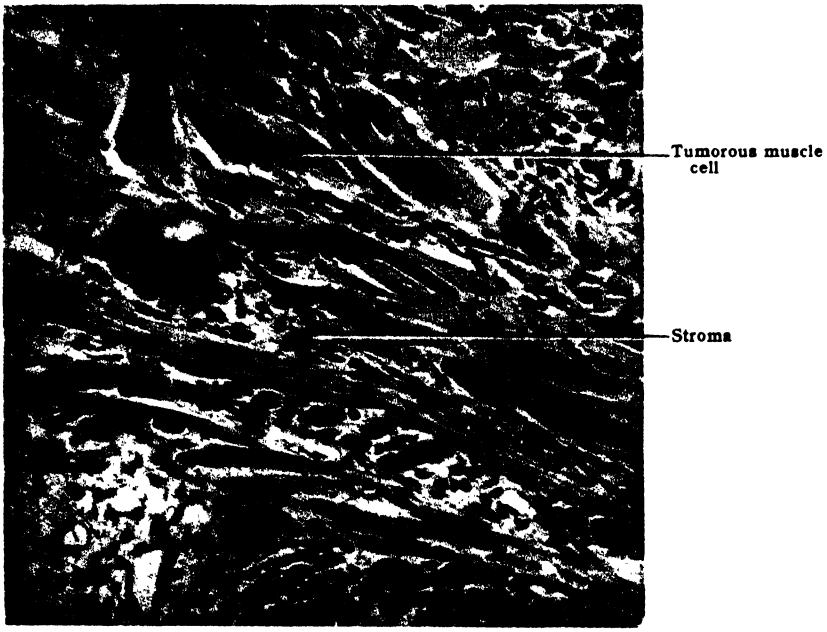


Fig. 114.—Rhabdomyosarcoma that originated in the striated muscle (×400).

There are usually some cells showing definite cross striations, as in normal muscle, but most of these cells resemble embryonic muscle. Nuclei are large, nearly round, fairly deeply staining and centrally located. The stroma consists of an abundant blood supply and a small amount of connective tissue. Where the cells are smaller and less differentiated, they become more like fibrosarcoma in cellular characteristics and general arrangement.

Infiltration of normal tissue occurs. Mitotic figures are not very abundant in the foci which are most like normal muscle. This tumor can be distinguished from fibrosarcoma of the connective tissue invading normal muscle, for in the latter, muscle is being destroyed, while in the former there are foci resembling embryonic and regenerating muscle cells.

Osteogenic sarcoma.—This is a malignant tumor of bone origin (Fig. 115). Primary tumors in the bone would include those originating from osteogenic tissue and those arising from the bone marrow cells. In this section we will consider only those which develop from osteogenic tissue and which retain, more or less, the ability to form bone. The *myelocytomas* or bone marrow tumors are considered in the section on the tumors of the blood forming and blood destroying tissues.

We have not found a large number of osteogenic sarcomas in our laboratory. However, they have occurred in scattered body regions including the skull, jaw, humerus, ribs, pelvis, femur and tail vertebrae. The stocks

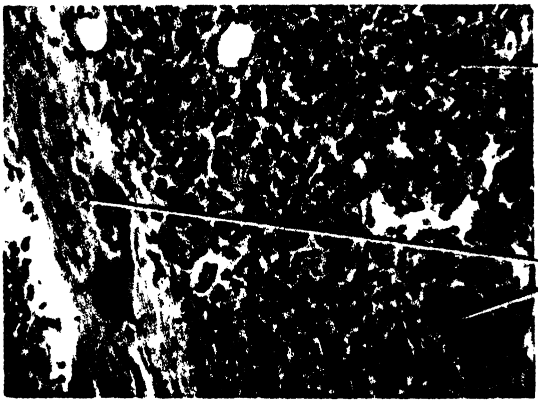


FIG. 115.—Osteogenic sarcoma from a rib (×200).

showing most of these osteogenic sarcomas have been the yellow, the C57 black, dba, X, Danforth's posterior duplication and Zavadskaia's brachyury. Hybrids of some of these stocks with other lines have also developed osteogenic sarcoma.

Pybus and Miller (45), on the other hand, were able to derive sublines of the Simpson strain of mice that develop a high incidence of spontaneous bone tumors. These developed at about sixteen months of age and originated in the skull, jaw, fore and hind limbs, ribs, sternum, pelvis and spine, most often in the last named site. Among the early reports on bone tumors were those by Ehrlich (10), Haaland (22), and J. A. Murray (43).

These tumors may develop as compact, rapidly growing, spindle-shaped cells resembling fibrosarcoma. However, most of these tumors retain their bone forming potentiality and show varying amounts of cartilage and bone in all stages of development. Cartilage may or may not be found, and frequently the tumors show foci of branching and anastomosing bony trabeculae. Surrounding the bone and cartilage are interlacing strands of

small, closely packed, spindle-shaped tumor cells and larger polyhedral tumor cells.

Mitotic figures are usually quite abundant in the spindle cells. Invasion into the surrounding normal tissues is quite extensive. Metastasis to the lungs has been observed in several cases and definite bone formation is seen in the pulmonary metastases.

When a sarcoma originating from bone shows definite cartilage formation without newly formed bone, it is called a *chondrosarcoma*. The bone sarcomas with some cartilage and considerable bone may be called *chondro-osteosarcomas*. It is convenient, however, to call all malignant bone forming tumors *osteogenic sarcomas*.

Angio-endotheliomas.—These are malignant tumors that appear to have arisen within some of the pre-existing benign hemangiomas and lymphangiomas due to the malignant changes which involved the endothelial cells. Malignant changes produce sarcomas which are called hemangio-endotheliomas and lymphangio-endotheliomas respectively.

In *hemangio-endothelioma* the general architecture of the hemangioma is evident. However, the endothelial cells are enlarged, rounded, vary in size and have invaded much of the stroma. There are foci of solid masses of cells in which the blood filled spaces have been obliterated. The cells have rather deeply staining, finely granular, eosinophilic cytoplasm and moderately hypochromatic oval nuclei. The nuclei contain many finely divided chromatin granules. Mitoses are often abundant. Invasion of adjacent normal tissues occurs and the simultaneous presence of this tumor in the leg and spleen has been observed. Whether this is a case of multiple primaries or metastasis is not easily determined.

The *lymphangio-endothelioma* shows the same type of malignant endothelial cells invading the stroma. These tumor cells also grow into the endothelial lined spaces. Infiltration of adjacent normal tissues is seen. Mitoses may be frequent.

Tumors of the Lung

There have been several publications (4, 20, 50 and 67) on tumors of the lung, but probably the first was by Livingood in 1896 (36).

The primary tumors of the lung are mainly those originating from the lining cells of the bronchi and the alveoli. They may be classified as:

1. Adenoma.
2. Adenocarcinoma.
3. Papillary adenocarcinoma.

4. Carcinoma simplex.
5. Carcinosarcoma.

Workers have disagreed as to the degree of malignancy of lung tumors. Among our tumor slides there are a large number of cases of spontaneous tumors of the lung, many of which show characteristics definitely demonstrating the malignant nature of lung tumors.

Adenoma.—Some small tumors are classed as adenomas because of their cell arrangement and comparative inactivity, but small size alone is not a true indication of the mass being benign. We have not observed a well developed capsule, possibly because of the looseness of the lung architecture. Adenomas appear fairly frequently as subserous, pearly white, slightly elevated nodules, often one half to two millimeters in diameter. On section they may be lenticulate to round. Their histopathology shows rather closely packed, poorly staining, polyhedral cells whose arrangement as twisting, branching tubules with blunt ends is suggestive of immature, uninflated air cells. Between these poorly defined structures is a network of thin-walled, capillary-like blood vessels. The polyhedral cells are not markedly different from many of the lining cells of normal pulmonary alveoli. They have centrally placed, rounded or oval, somewhat hypochromatic nuclei with one or more nucleoli, and abundant pale cytoplasm filled with fine eosinophilic granules. The tumor cells differ from the normal in that some are twice as large as the normal cells, some have lobulated nuclei and others show two nuclei within a single cell. Not all of these tumors are at the surface but are most likely to be observed there in gross dissection.

Some other tumors no larger than the above may differ from them chiefly in that the parenchyma cells are more closely packed, have more eosinophilic cytoplasmic granules, and exhibit a preponderance of large irregular cells. These have hypochromatic nuclei which often appear as two distinct nuclei within a single cell. It is not uncommon to see nuclei with multiple lobules, and sometimes a dozen or more closely clustered, rounded nuclear masses are seen within a single tumor cell. This indicates amitotic division. Mitotic figures are also occasionally seen.

The outlines of these two types of tumors differ in that the latter may be more irregular. In this type there are some foci of infiltration into normal alveoli, while other foci show compressed, collapsed alveoli resulting from the pressure of the tumor growth by expansion. Invasion of the smaller normal bronchi can sometimes be seen. We call this type an adenocarcinoma.

Adenocarcinoma.—It is a common belief that this tumor originates from the lining cells of the alveoli but it may also originate from the bronchi.

Here the tumor consists of poorly formed, gland-like structures scattered through nests of irregularly arranged tumor cells. These cells vary from polyhedral to columnar, possess hypochromatic nuclei and abundant cytoplasm filled with fine eosinophilic granules which are similar to those of the epithelium of the bronchi. The stroma consists principally of thin-walled blood vessels. Mitoses are frequent. Metastases in the liver have been observed.

Papillary adenocarcinoma of the lung.—This type may be seen as definite masses within, or continuous with, the more benign adenomatous form discussed above, and apparently arises through malignant changes. This type of carcinoma may also be in direct continuity with one of the smaller bronchi. The papillary type of growth is by far the most commonly seen among our carcinomas of the lungs of mice. Even tiny masses show it as definitely as the large tumors, which may involve an entire lobe of the lung. The architecture shows closely packed, branching and anastomosing, thin, finger-like strands with a stromal core of capillaries and a varying amount, usually small, of connective tissue stromal cells (Fig. 116). The papillary adenocarcinomas are darker staining than the benign type, have more cells, many of which are larger and show piling up to form several cell layers at many foci on the papillae. The tumor masses are chiefly composed of papillary structures without cyst formation. Occasionally one can observe dense foci of connective tissue stroma from which several of the papillary growths extend to form the main tumor mass. The stroma in the bulk of the tumor is often scant. The tumor cells of papillary adenocarcinomas vary in size and shape. Eosinophilic cytoplasmic granules are prominent, nuciei are hypochromatic and vary in size and shape, with some multilobulated, bilobed and binucleated forms present. In some tumors, foci of columnar tumor cells show well developed goblet cells. A few tumors may exhibit one to several layers of epithelial tumor cells lining intercommunicating spaces with only thin strands of stroma between them. Into these spaces project short, branching papillae giving an irregular appearance to the lining. On cross section these structures appear as large glands, not as cysts, presenting frequent foci of ciliated columnar epithelial tumor cells. These are located between the more piled up foci of tumor cells.

Papillary adenocarcinomas are often found close to the smaller bronchi, and it is not uncommon for these tumors to extend into these bronchi, nearly occluding them.

Carcinoma simplex.—This may originate in a papillary adenocarcinoma and often appears as closely packed cells arranged in an irregular pattern.

This type contains very little stroma except for numerous small, endothelial lined, capillary-like blood vessels. The outlines of the tumor cells may be vague but suggest round and polyhedral shapes. They have irregular, oval, hypochromatic nuclei and the cytoplasm is filled with rather deeply staining

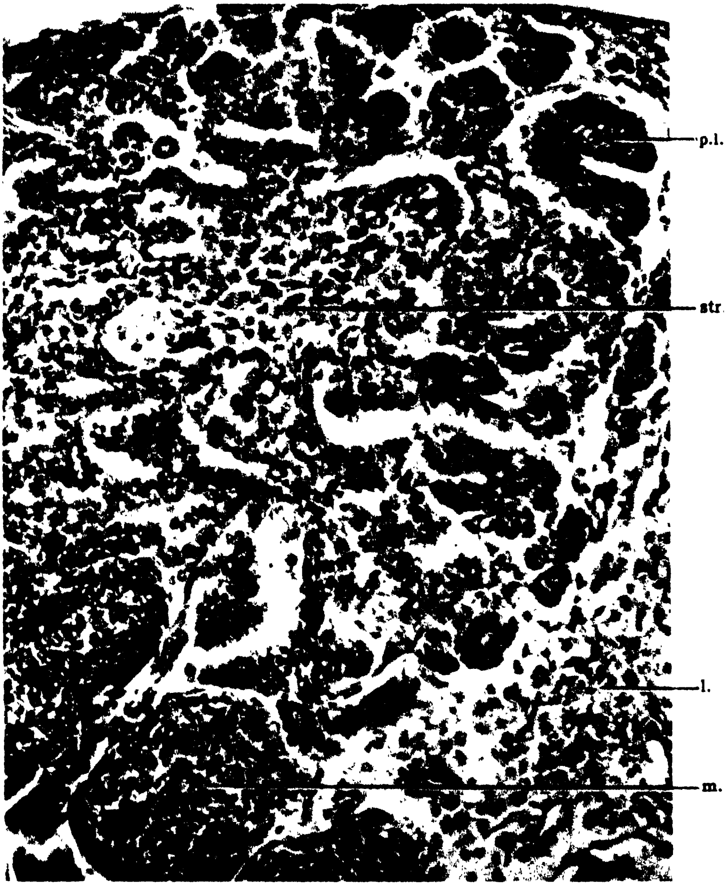


FIG. 116.—Primary papillary adenocarcinoma of the lung (×200). This tumor is sub-pleural and has been invaded by metastatic carcinoma of the mammary gland (lower left on the illustration). l., lung; m.m., metastatic mammary gland carcinoma; p.l., primary lung carcinoma; str., stroma.

eosinophilic granules. Sometimes the cytoplasm is reduced in amount, nuclei vary in size and have prominent nucleoli. These cells show no definite arrangement and are accompanied by more stroma than the above. The undifferentiated tumor cells may blend with definite papillary adenocarcinoma. This tumor shows abundant mitotic figures and may develop widespread metastases.

Carcinosarcoma.—This type may occur when the stromal connective tissue of a lung carcinoma becomes malignant. We have seen it most commonly in papillary adenocarcinomas. The sarcomatous part is composed of rather large spindle cells irregularly arranged in interlacing strands. Nuclei are spindle-shaped, darker than in the carcinoma, and the eosinophilic cytoplasm appears to have longitudinal striations but no granules. Mitotic figures are abundant.

Primary tumors of the lung other than the above types are rare. However, the lung is a common site for metastases of carcinomas and sarcomas from many other body regions (Fig. 116). This is especially true of carcinomas in the mammary region which grossly may resemble carcinomas primary in the lung. On histological examination a primary tumor of the bronchi or alveoli can be identified as such by the characteristics of the tumor cells. This includes their close resemblance to the normal lining cells of the bronchi and alveoli in cell outline and staining properties. Primary lung carcinoma cells are paler than mammary carcinoma cells and contain fine eosinophilic cytoplasmic granules, as well as lobulated nuclei and multinucleated cells which are not characteristic of the breast carcinomas. Other types of pulmonary metastases that have been seen are from carcinoma of the liver, malignant melanoma, osteogenic sarcoma, lymphocytoma, monocytoma, etc.

Tumors of the Blood Forming and Blood Destroying Tissues (Round Cell Sarcomas)

Enlargement of the mesenteric lymph nodes is not uncommon in old mice from many of our stocks. Frequently this enlargement is benign and is associated with some chronic infection. The usual finding in such cases is lymph node hyperplasia. There is, however, a tendency for a small percentage of the mice from nearly all of the stocks to develop spontaneous neoplasms of any of the lymph nodes, the spleen and sometimes the thymus. Occasionally a tumor appears at a single focus, such as the mesenteric lymph node. When the axillary and inguinal lymph nodes are involved, there is usually a bilateral enlargement of these glands as well as of the cervical lymph nodes. The spleen and internal lymph nodes may or may not become enlarged when bilateral enlargement of the subcutaneous lymph nodes occurs.

Mice with neoplasms involving the spleen and lymph nodes do not usually live long after the symptoms become marked. The outstanding gross characteristics are signs of ill health, such as dull, rough coat and

general emaciation accompanied by weakness and kyphosis. The abdomen becomes greatly distended by either enlargement of the spleen or ascites, or a combination of these two conditions. In some advanced cases marked subcutaneous edema obscures the emaciation. When the body cavity is opened, the edematous subcutaneous tissue is found to contain a clear serous-like fluid, and the intra-abdominal liquid may be serous or sero-sanguineous. Hydrothorax is also a fairly common finding when ascites is marked.

Lymph nodes are not uniformly enlarged. The mesenteric lymph node is usually but not always involved, and may be enlarged to three centimeters in length. Sometimes the spleen is enormous, light gray, friable and granular. Other lymph nodes may be enlarged in varying degrees. In a condition that is generalized the subcutaneous, mediastinal and intraperitoneal lymph nodes are enlarged and tumor cells from them show infiltrations into adjacent normal tissues and organs. The organs outside the lymphatic system which most frequently show gross involvement and tumor nodules are the liver, kidneys and the lungs.

The microscopic picture presented by these neoplasms is varied, since the tumors may be made up of cells which are predominantly from the unrestricted proliferation of lymphocytes, of immature myelocytes or of monocytes. These cells produce tumors with varying frequency in different stocks of mice. Numerous workers have published on this group of tumors in mice. Probably the earliest report was by Eberth (1878). However, many early reports were on small numbers of animals and the terminology employed has sometimes been confusing. More recently there have been several reports employing large numbers of mice and a fairly clear classification is in use. Tumors resulting from the unrestricted proliferation of lymphocytes and immature myeloid cells are well understood (Table 1). The third group, however, is less understood. This is largely because the origin and nature of monocytes are among the most debated problems of morphologic hematology. The interpretation used here is based upon the classification of human tissues employed in the Lymphatic Tumor Division of the American Registry of Pathology (6). From a review of the literature and from experiments conducted by himself and his co-workers, J. Furth (15) gives his conclusions on terminology and says: "that monocytes, histiocytes, macrophages, clasmatocytes, polyblasts, Kupffer cells and microglia cells are synonymous terms for one cell type, which is capable of perpetuating itself by mitotic division. In this (Furth's) communication we shall refer to the round forms of this type of cell seen in the circulating

blood, as monocytes, and to all other forms as histiocytes. Tumors of monocytes or histiocytes will be named histiocytomata (monocytoma) and the systemic disease characterized by these cells histiocytomatosis (monocytomatosis). Monocytic leukemia is a synonymous term for leukemic

Table 1

Tumors and Tumor-like Conditions of Blood Forming and Blood Destroying Tissues in Mice

Cells	Lymphocytes	Myeloid Cells		Monocytes (Histiocytes)
		Granular Leukocytes	Red Blood Corpuscles	
Non-neoplastic increase in cells	1. Hyperplastic lymph nodes (Lymphoma)	1. Extramedullary myelopoiesis	Polycythemia*	Granuloma (infections)
	2. Benign lymphoid infiltrations	2. Leukocytosis		
Neoplastic (invasive with cells fairly uniform)	Lymphocytoma 1. Leukemic 2. Aleukemic	Myelocytoma 1. Leukemic 2. Aleukemic	Erythrocytoma*	Monocytoma 1. Leukemic 2. Aleukemic
Neoplastic (invasive and cells pleomorphic)	Lymphosarcoma 1. Leukemic 2. Aleukemic	Myelosarcoma 1. Leukemic 2. Aleukemic	Erythrosarcoma*	Monocyte sarcoma 1. Leukemic 2. Aleukemic
Early site of neoplasm	Germinal centers of spleen and lymph nodes	Red marrow and outside germinal centers—red pulp of spleen and medullary tissue of lymph nodes		Histiocytes of liver, spleen and lymph nodes

* No cases on record in mice.

histiocytomatosis (monocytomatosis)." In consideration of the above we have employed the classification shown in Table 1.

Each of these types of tumors may or may not show an abnormal blood picture. When these tumors are accompanied by a marked increase of the tumor cells in the circulating blood, outside of the lymphatic system, this condition is called *leukemia*. The absence or paucity of these tumor cells in the circulating blood of animals that have developed tumors of this group is

called *aleukemia* (pseudo-leukemia). Without blood smears, it is not easy to classify these tumors as to whether they are leukemic or aleukemic. Some workers (48) have drawn their conclusions from a study of the large blood vessels of the liver, lungs and kidneys. Tissue imprints with special stains have been very valuable in determining the types of abnormal cells present in the tissues (33). Generally, the greater the number of tumor cells in the blood, the less the lymph nodes are enlarged and vice versa.

Most authors agree that there are no benign tumors produced by either lymphocytes, myeloid cells or monocytes. The non-neoplastic condition which has caused the most confusion is probably *non-malignant extramedullary myelopoiesis*. This condition is very common in the spleen of older mice of some stocks (3). In extramedullary blood forming foci all the elements of the normal marrow are usually present. The granulopoietic elements most often predominate over the erythropoietic and megakaryocytic elements. This condition is found most frequently in the spleen and liver. The sites usually involved by extramedullary myelopoiesis are similar to those in cases of myeloid leukemia. In the former all stages of development of myeloid cells are present, while in the latter most myeloid cells are immature. Additional information on the differences between these two conditions can be found under myeloid tumors.

Lymphocyte Tumors (Lymphoblastoma)

The most commonly observed tumors of the lymphatic system are those of the lymphocytes (Fig. 117). They appear first in the nodules of the lymph nodes and in the Malpighian bodies of the spleen. The primary foci increase in size, due to proliferation of the lymphocytes, and progress until they obliterate the normal architecture of the lymph nodes and spleen, leaving only uniform masses of lymphocytes. These tumors always invade the lymph node capsules (48). Due to the extent of the lymphatic system, infiltration of adjacent tissues is difficult to differentiate from true metastases.

Lymphocytoma.—This shows fairly uniform cells of the lymphocyte type; however, they are larger than normal cells. Usually they belong to the large lymphocyte variety. Mitotic figures are often numerous (Fig. 117). The liver, lungs and kidneys are the organs most often invaded. In the liver the periportal foci are first involved, in the lungs perivascular infiltration is most marked and in the kidney the infiltration extends inward from the hilus. This tumor may be leukemic or aleukemic, focal or more or

less generalized. In the generalized type there is usually a focus of greatest lymph node enlargement.

Lymphosarcoma.—(Lymphoblastoma sarcoma type.) This type may be leukemic or aleukemic. The cells are more pleomorphic than in lymphocytoma, showing atypical lymphocytes with irregular nuclei and little cytoplasm. The more atypical the cells the more malignant the tumor. Mitoses are abundant.

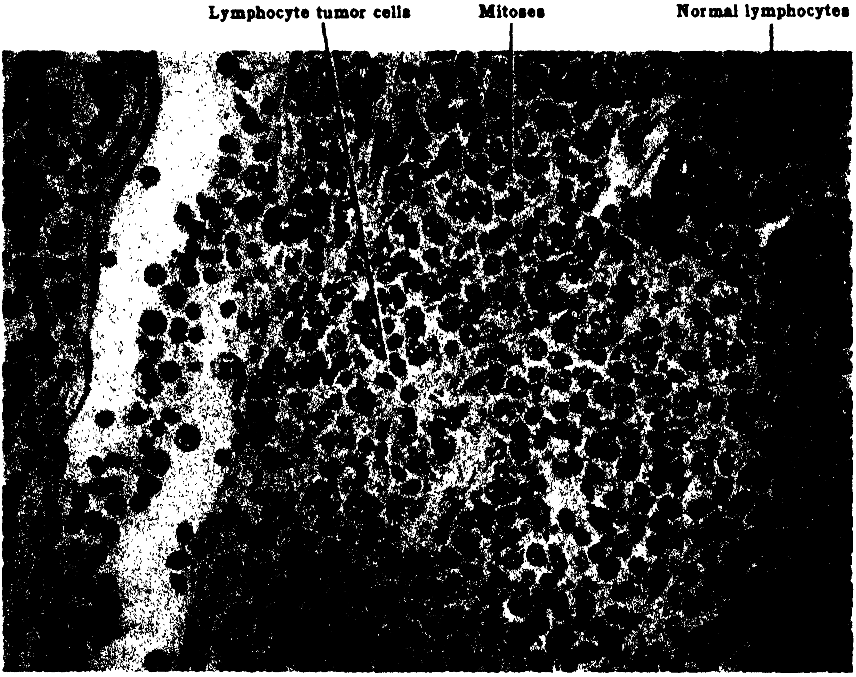


FIG. 117.—Lymphocytoma in the mesenteric lymph node (×400).

MYELOID CELL TUMORS

Only those tumors from myeloid cells which form the granular leukocytes will be considered. These tumors are rare in most stocks of mice, but Barnes and Sisman rep)rt that several cases have been seen in their stock Rf and in stock S, and the same is true of Strong's F strain (3, 33). The sites of early involvement are bone marrow, the red pulp of the spleen and the medullary tissue of the lymph nodes. Lymph nodes are sometimes greenish (chloroma). In the spleen and lymph nodes the immature myeloid cells surround the germinal centers and may obliterate them. The following criteria have been suggested as an aid in distinguishing between myeloid cell tumors and *non-malignant extramedullary myelopoiesis* in mice (3).

Myeloid Cell Tumors	*Non-Malignant Extramedullary Myelopoiesis*
Most myeloid cells are immature	All stages of development of myeloid cells are present
Erythrogenic foci are absent among myeloid cells	Erythrogenic foci are usually present
Megakaryocytes are few and present only in the organs (the spleen, liver and lymph nodes) where they are found in non-leukemic conditions	Megakaryocytes are usually numerous
Myeloid cells often invade muscle and other non-hematopoietic tissues	Cells are non-invasive
Blood usually contains *immature* myeloid cells	Blood is normal or there is leukocytoses with numerous *mature* forms
Liver is usually enlarged and gray-brown	Liver is usually not enlarged and is brown-red
Most of the lymph nodes are usually enlarged	Most of the lymph nodes are usually of normal size
Hemorrhages are frequent in viscera (lungs, lymph nodes, etc.)	Hemorrhagic manifestations are absent
Transmissible to other mice	Not yet shown to be transmissible
Not shown to be produced by bacteria	Can be produced by bacteria

Myelocytoma.—This is a tumor made up of immature myeloid cells. The predominating tumor cells may be myeloblasts or different kinds of immature granulocytes (Fig. 118). There may be considerable variation between the cells of different tumors, but the cells have a tendency to be rather uniform in individual cases. Variations in size and staining power of these tumor cells indicate an approach to the sarcoma type. Myelocytoma may be leukemic or aleukemic. Mitoses may be fairly frequent and invasion into adjacent normal tissues occurs.

Myelosarcoma.—This is similar to myelocytoma, except that the cells are more variable in size and assume bizarre shapes. Mitoses are frequent. Extensive infiltrations into normal tissues may occur.

MONOCYTE TUMORS (MONOCYTOMA OR HISTIOCYTOMA*)

These tumors are rare in many stocks of mice. However, Tyzzer (67) and Slye (51) report cases, without diagnoses, which from their pictures and descriptions appear to be similar to a type of tumor (probably monocytoma)

* (The neoplasms sometimes called reticulum cell tumors probably belong in this group.)

seen in the C57 black, C57 brown and C57 leaden stocks. There are occasional reports in the literature on reticulum cell tumors and references to cases in mice resembling Hodgkin's disease (15, 26). J. Furth states that most human neoplasms of the histiocytes have been described as reticulum cell sarcoma or reticulosis, leukemic and aleukemic, but since the relation of histiocytes to reticulum fibers and to reticular fibroblast-like cells of blood forming organs is obscure, this terminology is not desirable. He suggests the use of the term *histiocytoma* or *monocytoma* to cover these tumors in the blood forming organs.

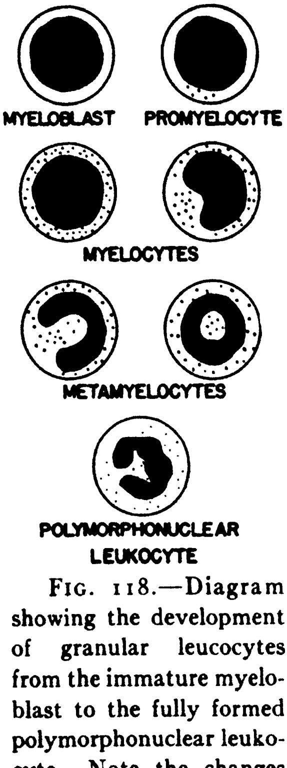


FIG. 118.—Diagram showing the development of granular leucocytes from the immature myeloblast to the fully formed polymorphonuclear leukocyte. Note the changes in number and size of the granules. (*Redrawn from Barnes and Sisman.*)

Monocytoma.—Monocytes may form tumors in the liver and spleen and infiltrate these and other tissues. The liver becomes enormously enlarged and mottled with minute irregular gray-white masses of tumor cells, hemorrhagic areas and yellowish-gray necrotic foci. The spleen and lymph nodes may or may not be enlarged grossly. Microscopically the spleen usually shows small foci of tumor cells, hemorrhage and necrosis. Occasionally the lungs show large or small areas of hemorrhage with yellowish-gray foci of tumor cells. Death usually results from internal hemorrhage starting from the lesions in the liver.

There are leukemic and aleukemic forms of this tumor. The cells are the large monocyte type with oval, bean-shaped or irregularly lobed, eccentric nuclei. Considerable non-granular basophilic cytoplasm is present. Infiltration of the liver is diffuse with frequent formation of small tumor nodules. Malignant cells are seen within the blood vessels of the liver, sometimes nearly occluding them. The spleen may show the same type of tumor cells and blood vessel involvement. Lymph nodes may or may not be involved. When involvement occurs, it is around the nodules. Mitoses are somewhat numerous. The tumor cells have some phagocytic ability. A condition exactly like Hodgkin's disease has not been found in mice, but there are certain similarities between monocytoma and Hodgkin's disease (15).

Monocyte sarcoma.—This tumor is similar to the above but mitoses are more frequent and the cells show more variation in size. Large tumor giant cells and bizarre shaped cells are often seen.

The writer is well aware that there is considerable difference of opinion concerning the monocyte tumors. Because of this the above discussion is necessarily brief and somewhat incomplete. The work now being done at several institutions should be of real value in clarifying their classification and nomenclature.

Tumors of the Digestive System and Associated Glands

Digestive Tube and Submaxillary Gland Tumors

Tumors of this region are rare but they do occur. In the submaxillary gland we have seen an *adenoma* in a yellow stock mouse and a *carcinoma simplex* in an A stock albino female. Tumors of the oral cavity and the esophagus have been extremely rare (68). However, we have had a *papilloma* develop in the esophagus of a mouse.

Marked hyperplasia of the epithelium in the glandular part of the stomach has been observed in several of our mice. This has also been recorded in other laboratories. Wells (68) reviews the literature and reports three *adenocarcinomas* of the pylorus. *Epidermoid carcinoma* of the non-glandular part of the stomach has been seen. Here the normal lining is stratified squamous epithelium and the tumor shows definite epithelial pearl formation. In a C57 black mouse this type of tumor has been observed to infiltrate through the stomach wall and to begin the invasion of the pancreas. Similar tumors have been recorded by Slye (54, 68) and others.

A few *intestinal polyps* have occurred. In one case beginning adenocarcinoma was observed in this type of polyp. Carcinomas are rarely observed, even though the intestine is inspected routinely at autopsy. In Slye's laboratory a few *squamous cell carcinomas* and *adenocarcinomas* of the intestine have been found (68). *Cavernous hemangioma* and primary *fibrosarcoma* have been found in our C57 black, the dba mice and their hybrids. Other fibrosarcomas have been seen in the mesentery of the small intestine. Lymphocyte tumors also occur in the lymph nodes of the intestines in several stocks.

Hepatic and Gall Bladder Tumors

The liver is a relatively common site for primary tumors (hepatomas). Regeneration adenomas are the result of rapid proliferation as an attempted repair following injury. Such lesions have not been classified as tumors.

True *adenomas* of the liver parenchyma cells occur most frequently in the C57 black, yellow, and dba stocks but are not limited to them. These tumors are circumscribed growths of atypical parenchyma cells with atypical arrangement but without signs of infiltration or marked activity. Slye (51) found a few similar tumors. *Carcinoma* of the liver is encountered quite often among our primary liver tumors. The usual picture is of large and small liver parenchyma cells growing in wild confusion with frequent tumor giant cells and bizarre cell forms (Fig. 119). Normal architecture is lost and

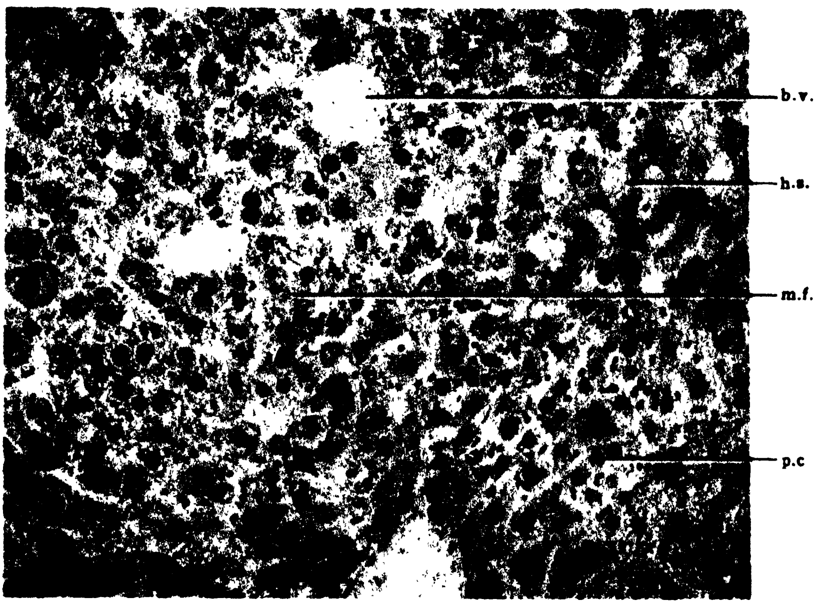


FIG. 119.—Carcinoma of the liver parenchyma cells (×200). b.v., blood vessel; h.s., hepatic sinusoid; m.f., mitotic figure; p.c., tumorous liver parenchyma cells.

invasion of adjacent normal liver occurs. Mitoses may be frequent and metastasis to the lung occurs. The tumors of the liver parenchyma cells appear grossly as elevated or pedunculated masses that are almost the same color as normal liver. There is a rare form of carcinoma of the liver composed of large, pale cells whose arrangement suggests attempted gland formations. Mitoses are frequent. *Papilloma* of the gall bladder has been observed.

Non-epithelial liver tumors are also seen somewhat frequently. Tumors of the lymphoid, myeloid, and monocyte cells are taken up elsewhere. *Hemangiomas* are a fairly common type of liver tumor, and *hemangio-endotheliomas* are occasionally observed in some stocks. A *lymphangio-*

endothelioma has been seen in the liver of an A stock female. *Fibrosarcomas* of the liver have been observed in several stocks.

Pancreatic Tumors

Primary tumors of the pancreas are rare. *Adenocarcinoma* has been observed in one *Mus bactrianus* female and in two hybrids of this stock crossed with C57 black. Slye (61) reported two cases in 125,000 autopsies.

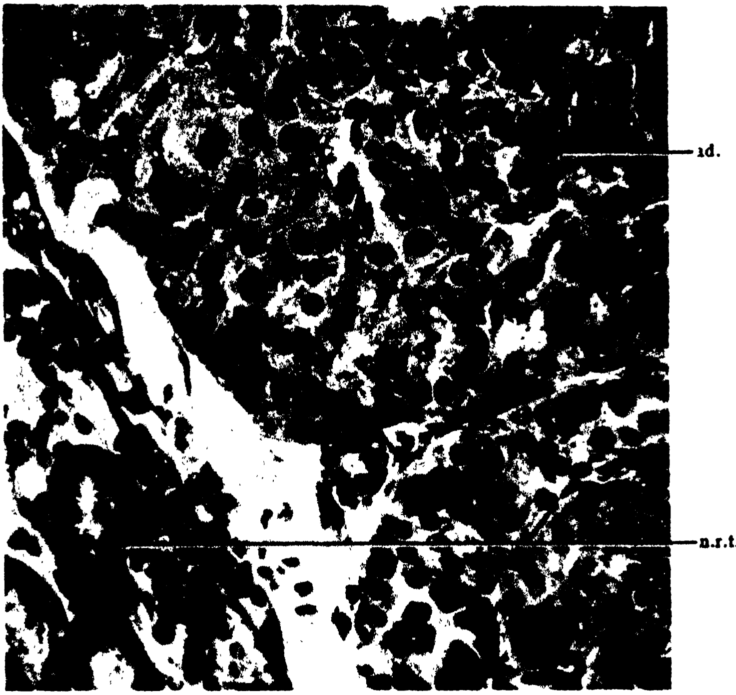


FIG. 120.—Adenoma of the renal tubules (×400). ad., adenoma; n.r.t., normal renal tubule.

Carcinoma of the pancreatic islands has been seen twice in our stocks. Hueper (27) reports another case. *Fibrosarcoma* also occurs in this gland.

Tumors of the Uro-genital System

Kidney and Urinary Bladder Tumors

The kidney is a fairly frequent site for secondary involvement of tumors of the blood forming and blood destroying tissues. However, primary tumors of this organ are not common. We have had one case of *adenoma* of the renal tubules (Fig. 120) and one case of *papillary cyst adenocarcinoma*. Several *papillomas* of the renal pelvis have occurred (Fig. 121A). A few of

these papillomas showed beginning *adenocarcinoma* (Fig. 121B). Primary *fibrosarcoma* also occurs in this organ. Slye (57) reported on kidney tumors of several types.

Although tumors of the urinary bladder are extremely rare, *papillomas* have been found. One extensively invading and rapidly growing *carcinoma*

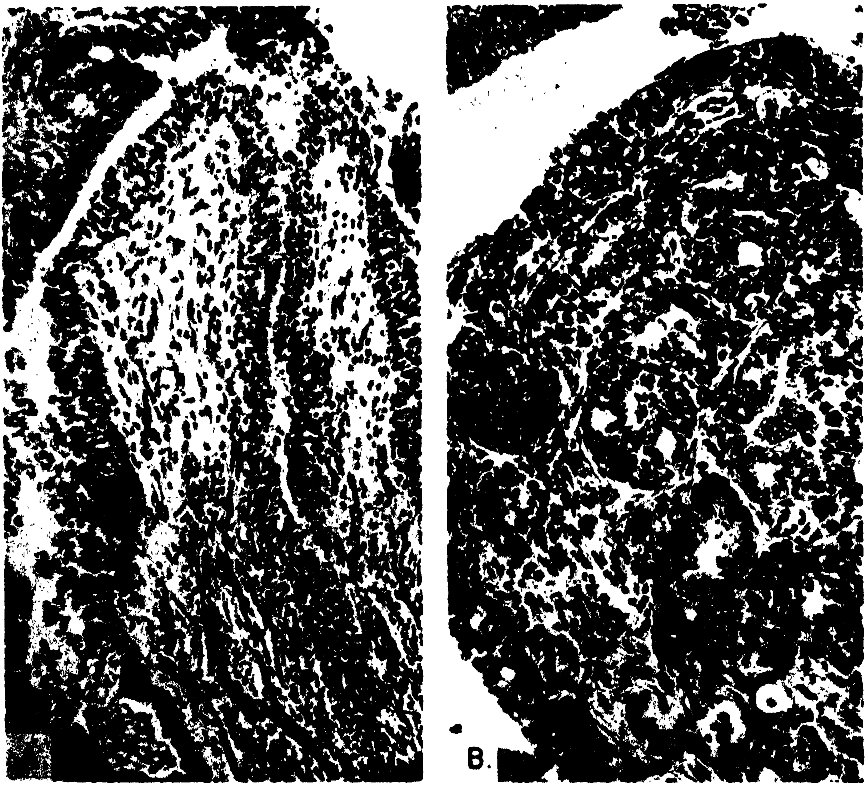


FIG. 121.—Tumors of the renal pelvis. A, papilloma of the renal pelvis (×200); B, adenocarcinoma in a papilloma of the renal pelvis (×200).

of the transitional cell epithelium occurred in a Riga stock female. *Hemangiomas* are also seen (Fig. 111A).

Ovarian Tumors

Among the first reports on tumors of the ovaries in mice were those of Jobling (30), Tyzzer (67) and Haaland (23). Slye, Holmes and Wells (56) stated that they had found 44 primary ovarian tumors in 22,000 autopsies. Gardner, Strong and Smith (19) described a case of spontaneous bilateral granulosa cell tumors in an old mouse. J. Furth and Butterworth discuss

the types of ovarian tumors found in mice subjected to x-rays and state that spontaneous tumors of the ovaries are very rare (16). This last paper is of interest to us in that we have observed, in our spontaneous tumors of the ovaries, types that compare with most tumors which developed following irradiation.

Probably because of the complexity of the ovary it can be the primary site of a fair number of tumors in some stocks of mice. This is true of the ce (extreme dilution) stock in our laboratory. Scattered cases have been found in several of the pure stocks. Also offspring of crosses between pure stocks have developed several spontaneous tumors especially where the C57 black mice have been crossed with the dba, A albinos and *Mus bactrianus*. Altogether we have found over 50 spontaneous ovarian tumors in our mice. This does not include *simple cysts* which are common and are not malignant. They are probably associated with abnormal physiology. These cysts may be lined by a single layer of flattened or cuboidal epithelium and occasionally are distended by hemorrhage into the cyst cavities. Those lined by ciliated cuboidal epithelium arise from the vestigial tubules, the epoophoron.

Ovarian tumors in mice show considerable variation in appearance. The majority of these tumors already observed would probably fit into one of the following classes. No doubt additional forms will be found.

A. Cystic tumors.
 Papillary cyst adenoma.
 Papillary cyst adenocarcinoma.

B. Solid tumors.
 Granulosa cell tumor.
 Hemangioma.
 Hemangio-endothelioma.
 Fibroma.
 Fibrosarcoma.

C. Embryonal tumors.
 Teratoma.
 Embryonal adenoma.
 Embryonal cell carcinoma.

Cystic tumors.—A cyst that grossly appears to be simple may contain numerous small or large papillary ingrowths. These papillae may have simple cuboidal or columnar epithelial cells covering their surfaces (Fig. 122). Such tumors are ***papillary cyst adenomas*** and are benign. A ***papillary cyst adenocarcinoma*** may arise in the same manner.

Solid tumors.—In the non-tumorous ovary the changes of the follicular cells into granulosa cells and their further differentiation to form lutein cells are not sharp but gradual. For this reason definite lines cannot be drawn, and borderline cell types can be observed. Therefore, there is justification for considering that the tumors made up of cells resembling the above phases should be grouped together. The growing tendency is to call all such tumors *granulosa-cell tumors*. This type is the most common of

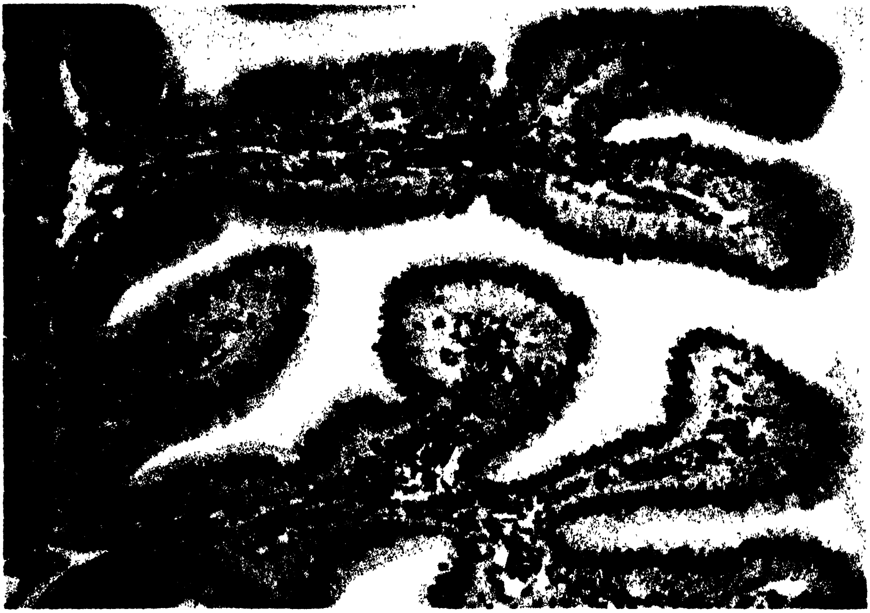


FIG. 122.—Papillary cyst adenoma of the ovary (×200).

the solid ovarian tumors observed here. Grossly they are usually rather large.

This places in one group tumors that show a wide variation in the type and arrangement of cells. The cells may be fairly uniform and rather closely resemble follicular, granulosa, theca or lutein cells. However, there is often what appears to be a mixture of two or more of these cell types. The cells may be arranged in a pattern that resembles closely packed, large and small follicles, some distinct and some confluent, separated by thin septa of stroma. Sometimes the cells grow in irregular cords which bear a resemblance to the trabeculae seen in the early stages of corpus luteum formation. There may also be seen more or less solid masses of cells with some stroma and scattered, almost gland-like foci that resemble attempted follicle formations. There are other tumors with large, pale, spindle shaped

cells which show foci that appear almost sarcomatous. Probably at least part of the latter are from theca interna cells. In the ce stock, at least, large clusters of Sertoli-like cells are often encountered with the last mentioned form of tumor cells. The large, clear, lutein-like cells seen by J. Furth and Butterworth (16) have not been found as the type cell of any of our spontaneous ovarian tumors. However, MacDowell-Bagg stock albinos treated with x-ray have produced several, and these have shown occasional mitotic figures. This is mentioned because the potentiality for the formation of lutein-like tumor cells is present and these tumors will probably appear spontaneously in rare cases.

All the above variations of spontaneous granulosa-cell tumors are probably benign. There are, however, mitotic figures in some cases, and the tumor masses may be fairly large and nodular in outline. Some sarcoma-like tumors have foci that suggest granulosa-cell tumors. These are difficult to diagnose with certainty.

Cavernous hemangioma is occasionally seen in the ovaries of mice. Still more uncommon is *hemangio-endothelioma* which has been observed a few times. True *fibrosarcoma* of the ovary is also rare in our stocks. A few of these tumors have been diagnosed as primary at this site. Fibromas have not been observed in our mice.

Embryonal tumors of the ovary.—A rare, benign tumor of the ovary is the *teratoma*. This usually shows a mixture of bone, cartilage, striated muscle and gland structures as well as other tissues. There may be skin, nerve or almost any tissue in this type of tumor (Fig. 123).

Occasionally there is a tumor composed of closely packed epithelial cells arranged as in embryonic gland formation. This is called an *embryonal adenoma* and is benign. The cells are uniform, small and deeply staining. Mitoses are rare.

Embryonal cell carcinoma is composed of large, rounded, pale epithelial cells varying in size. They have a fair amount of pale cytoplasm and rounded, hypochromatic nuclei with coarse chromatin granules. These cells are compactly arranged without much stroma; mitoses are abundant.

Uterine Tumors

Epithelial tumors at this site are rare in mice (58). Our records show that *adenomas* have been observed twice in the dba stock. *Carcinoma simplex* has also been observed in two mice, both hybrids, one from a cross between dba and C57 black, the other from a cross of dba with yellow. The former is shown in Figs. 124A and 125. Here the epithelium can be seen

grading over into carcinoma simplex tumor cells which are invading the uterine wall.

Of the non-epithelial tumors *fibrosarcoma* is the most common tumor of the uterus (Fig. 124B). Quite a number of cases have been seen. This, however, does not represent a high incidence in any of the pure stocks or



FIG. 123.—Teratoma of the ovary (×200). bn., bone; cart., cartilage; gl., gland; mu., striated muscle.

their hybrids. Of the pure stocks it is probably most common in the dba. It is, however, seen in the C57 black mice. Most of our cases have developed in crosses between these two stocks or in hybrids between C57 black and A albino.

With this tumor the uterus is greatly enlarged, firm and friable. The enlargement is usually bilateral and these tumors are not multiple as is the case of the fibroid tumors in the human.

Histologically this tumor is composed of small, closely packed, short spindle cells with little stroma. The cells are arranged in an irregular interlacing pattern of whorls (Fig. 124B). Mitotic figures are not common but

blood vessel invasion occurs and metastases in the liver are seen. The ovaries are sometimes involved by extension of this tumor.

Leiomyosarcoma is a tumor which is grossly like fibrosarcoma but microscopically is composed of larger, longer spindle cells. These cells are arranged in irregular interlacing bundles and are of the smooth muscle type characteristic of the uterine wall. Mitoses are not frequent.

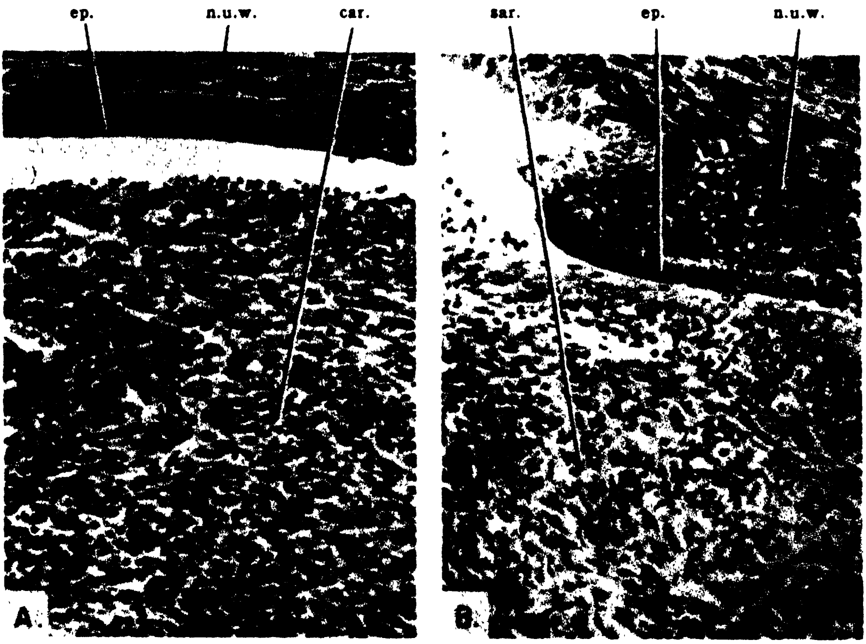


FIG. 124.—Primary tumors of the uterus. A, carcinoma simplex that originated in the uterine epithelium and invaded the uterine wall (×200); B, fibrosarcoma in the wall of the uterus (×200). car., carcinoma simplex; ep., epithelium; n.u.w., normal uterine wall; sar., fibrosarcoma.

Other tumor forms seen include adenofibrosarcoma, hemangioma and hemangio-endothelioma. All these are rare. The first shows a few uterine glands deep within a fibrosarcoma. The third type has been seen but once and was in the oviduct.

TUMORS OF THE TESTES

Tumors are rare at this site. Slye (55) reported 28 primary tumors in the testes of mice. The majority of her tumors appear to be similar to two cases that we have called *embryonal cell carcinoma*. These tumors show many of the characteristics described by her. The tumor cells are large, rounded and pale with abundant cytoplasm and hypochromatic nuclei

containing coarse chromatin granules. Some nuclei are vesicular. Mitotic figures are quite numerous. The architecture shows cords and dense masses of epithelial cells without much stroma. One of our cases appeared in the I stock and the other in the black-eyed white (AMC) stock.

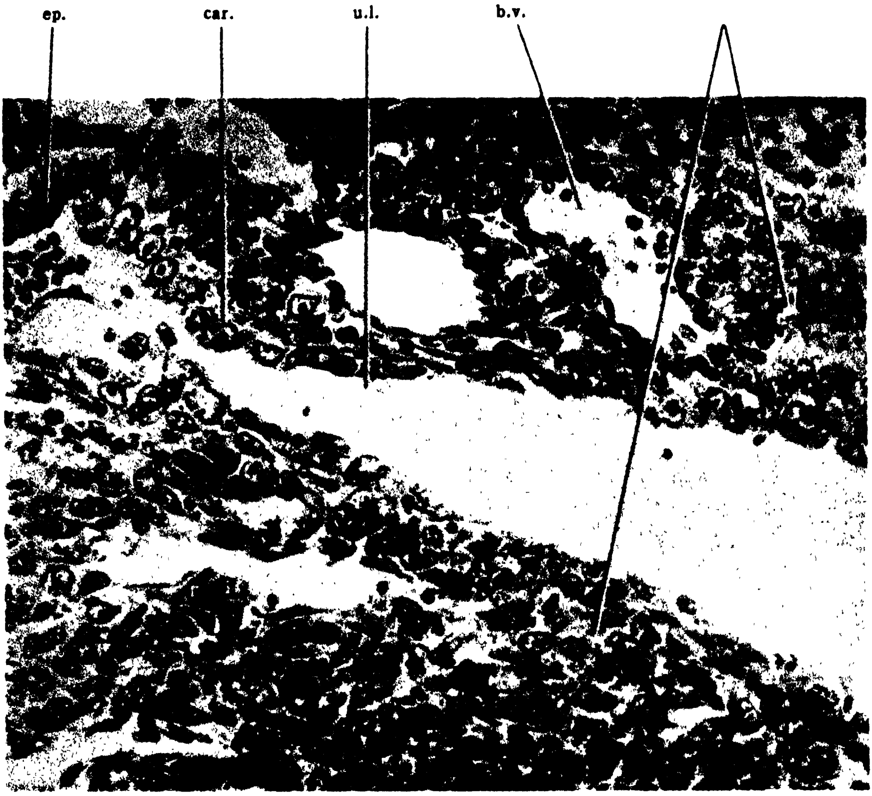


FIG. 125.—Carcinoma simplex of the uterus (×400). b.v., blood vessel; car., carcinoma simplex; e.p., epithelium grading over into carcinoma; u.l., uterine lumen.

No other types of tumors of the male reproductive organs have been found in our stocks. Slye reported sarcomas found in the testicle and in the seminal vesicle.

TUMORS OF THE CENTRAL NERVOUS SYSTEM

Brain tumors.—These neoplasms are rarely found in mice. We have observed a *medulloblastoma* (Fig. 126). Another tumor has been diagnosed as a *glioma*. Both of these were in C57 black females. It is of interest that in this same stock hydrocephaly has been observed in a number of young of

both sexes. The only other cases in the literature are a *papillary adenoma* of the ependyma cells of the lateral ventricle, an *endothelioma* of the cerebrum and an *adenoma* of the hypophysis in 11,118 autopsies by Slye (60). One other adenoma of the hypophysis has been reported (19). In our stocks we have diagnosed two *adenocarcinomas* of the hypophysis composed chiefly



FIG. 126.—Medulloblastoma from the brain of a mouse (×200).

of eosinophile cells. These were in hybrids from a cross between the C57 black and C57 brown stocks.

Other Rare Sites of Tumors

Among the rare sites of tumors is the heart. We have observed a rhabdomyosarcoma of this organ and Hertzog (25) reported a papillary fibroma of the cardiac valve. Slye (59) reported tumors of the thyroid. However, we have not observed neoplasms at this site. In the glands around the eye we have found two papillary cystadenomas, a papillary adenocarcinoma and an adenocarcinoma.

This chapter has been intended to emphasize, mainly, the types of spontaneous tumors that are most commonly encountered. As data on

spontaneous tumors are being steadily accumulated, there will be additional types of tumors found and more information will be available on the tumors at other sites.

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Chapter 5

GENE AND CHROMOSOME MUTATIONS

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Gene Mutations

In the following list of gene mutations are given (*1*) the symbol for the mutation adopted by the International Committee on Mouse Genetics Nomenclature; (*2*) the name of the mutation; (*3*) if a linkage is known, (*a*) the number of the chromosome to which the linkage group has been assigned, (*b*) the per cent of crossing-over between the known genes on this chromosome, (*c*) a reference to an article giving linkage data; (*4*) a brief description of the mutation; (*5*) a statement as to the dominance shown by the mutation (if no statement is made about dominance it may be assumed that any mutation represented by a small letter is completely recessive to its normal or type allele); (*6*) a reference to one or more important, and if possible recent, articles describing the mutation.

A agouti.—Chromosome 5. Linked with *pa* (20% crossing-over). Roberts and Quisenberry, 1935, Am. Nat. 69: 181–183. The normal or type allele of the agouti locus.

a non-agouti.—*aa* mice are solid black, lacking the sub-apical yellow band on each hair which gives wild-type mice their characteristic brownish color. In most stocks *a* is completely recessive to +, but strains have been reported in which +*a* mice have a dark or black back merging into nearly typical agouti on the sides and belly. In these strains ++ mice are only slightly darkened. There is some evidence that one principal partly dominant modifying gene, *umbrous* (*U*), is involved. Mather and North, 1940, J. Genet. 40: 229–241.

a^t *black-and-tan.*—Allele of *a*. Mice of the constitution a^ta^t and a^ta have a light belly (dirty yellow to white) and a black back. The line of demarcation between light and dark regions is quite sharp. Aa^t mice have a light belly and an agouti back. Dunn, 1916, Am. Nat. 50: 664–675.

A^w *light-bellied agouti.*—Allele of *a*. Like a^t except that the back is agouti instead of black. Morgan, 1915, Am. Nat. 49: 379–383.

A^y *lethal yellow.*—Allele of *a*. Mice heterozygous for A^y have a bright yellow coat and show a tendency to put on fat. Homozygous A^yA^y mice are non-viable, dying in the early stages of development. Little, 1919, Am. Nat. 53: 185–187. Kirkham, 1919, J. Exp. Zool. 28: 125–135.

ac absence of corpus callosum.—Brain lacks corpus callosum. Behavior normal. Keeler, 1933, Proc. Nat. Acad. Sc. 19: 609–611.

b brown.—Coat color cinnamon brown in combination with agouti, chocolate in combination with non-agouti. Completely recessive except in the genotypes *sisi* and *pp* in which it is partially dominant. Little, 1913, Carnegie Instn. Wash. Pub. No. 179: 11–102.

C color.—Chromosome 1. Between *sh-1* (3.1% crossing-over) and *p* (17.3% crossing-over). Grüneberg, 1936, J. Genet. 33: 255–265. *C* is the type allele of the albino series.

c albinism.—No pigment in coat or eyes. Recessive to *C* except that *Ccpp* mice are lighter than *CCpp* mice. Dunn, 1936, J. Genet. 33: 443–453.

c^e *extreme dilution.*—Allele of *c*. Coat quite light, eyes black, yellow pigment suppressed. Recessive to *C*, partly dominant to *c*, c^{ch}, and c^i. Detlefsen, 1921, Am. Nat. 55: 469.

c^{ch} *chinchilla.*—Allele of *c*. Coat lightened, but much less than by c^e, eyes black, yellow pigment suppressed. c^{ch} has more effect on the coat of *A* mice than of *aa* mice. Thus in *aaB* mice separation of $c^{ch}c^{ch}$ from *C* is sometimes difficult, although with practice $c^{ch}c^{ch}$ may be distinguished by the lesser saturation of black which tends to be dull and slate colored near the base of the fur, and especially by the shade of the hairs lining the ears which in *C* forms are yellowish, in $c^{ch}c^{ch}$ creamy or nearly white. Again, in *aabb* mice the genotypes *C*, $c^{ch}c^{ch}$ and $c^{ch}c^e$ are indistinguishable, whereas if *A* is present they can be separated. Recessive to *C*, partly dominant to *c*, c^e and c^i. Dunn, 1936, J. Genet. 33: 443–453.

c^i *intense chinchilla.*—Allele of *c*. Similar to c^{ch} but causes somewhat less lightening of the coat. Feldman, 1935, J. Mammal. 16: 207–210.

Ca caracul.—Chromosome *6*. Linked with *N* (1.8% crossing-over). Cooper, 1939, J. Hered. 30: 212. Vibrissae curled and coat wavy. Recognizable at one day after birth by slight curling of vibrissae. Less pronounced in old mice than in mice with the first pelage but easily recognized by the curled vibrissae and the waviness of the guard hairs. Very similar to *Re*. Completely dominant to type. Carnochan, 1937, J. Hered. 28: 333–334.

d dilution.—Chromosome 2. Linked with *se* (.06% crossing-over). Snell, 1931, Genetics 16: 42–74. The coat is diluted to a blue-gray or leaden color. In *aa* mice the color is similar to that of a Maltese cat. The pigmentation of the eyes at birth, as seen through the unopened eyelids, is slightly lighter than in *D* mice. Little, 1913, Carnegie Instn. Wash. Pub. No. 179: 11–102.

dw dwarf.—Causes practical cessation of growth at 14 days. Prior to this age there is some retardation of growth, so that by 7 days *dwdw* mice can usually be recognized by their smaller size. Sterile in both sexes. Due to pituitary deficiency. De Beer and Grüneberg, 1940, J. Genet. 39: 297–300.

f flexed tail.—Tail flexed due to fusion of vertebrae, newborn young anaemic, often accompanied by white spot on belly. The anaemia is due in part to a deficiency in the number of erythrocytes; more important however is a deficiency in the total amount of haemoglobin. It largely disappears at two weeks. The flexed tail condition is recessive with some normal overlaps, perhaps sometimes dominant. Mixter and Hunt, 1933, Genetics 18: 367–387.

gl grey-lethal.—Homozygous *glgl* mice, otherwise of wild phenotype, have a pure grey coat without a trace of yellow. In *aa* or c^ec^e genotypes, the *gl* gene produces little effect on coat color. There is a major effect on growth. *glgl* mice are slightly smaller than normals from birth to 14 days, thereafter they loose weight. The teeth do not erupt, their shape is abnormal and the roots uncalcified. The long limb bones are abnormal. Death occurs usually between the 22nd and 30th day. Grüneberg, 1938, J. Genet. 36: 153–170.

hr hairless.—Chromosome 3. Linked with *s* (about 9% crossing-over). Snell, 1931, Genetics 16: 42–74. Homozygous *hrhr* mice develop normally until about 14 days of age when, at just about the same time that the eyes open, they can be distinguished from normal sibs by loss of hair on the upper eyelid. At about the same time shedding begins on the under jaw and on all four feet just back of the toes, and slightly later at the base of the tail. During the next week shedding spreads from these centers, especially that around the eye, until the animal is naked except for a few scattered hairs. The vibrissae usually remain. There is sometimes a very slight regeneration of hair at about six weeks. Females are poor breeders or often completely sterile. David, 1932, Z. Zellforsch. u. mikr. Anat. 14: 616–719.

hr^{rh} rhino.—Allele of *hr*. At 13 or 14 days hair begins to shed above eyes just as in *hrhr* mice, but there is less definite anterior-posterior progres-

sion of shedding, hair tending to thin out all over. Also hair persists on feet as late as seventh week instead of falling out at 2 weeks as in *hrhr* mice. At about 3 weeks $hr^{rh}hr^{rh}$ mice begin to show a wrinkling of the skin which becomes very pronounced in old animals, giving "rhinoceros" appearance. Recessive to *hr*. Howard, 1940, J. Hered. 31: 467–470.

hy-1 hydrocephalus-1.—The lateral and third ventricles and the foramina of Monroe are distended with accumulated fluid. The aqueduct of Sylvius is occluded. The swelling of the head may be detectable at birth but usually does not become noticable until a week or two later. Affected mice become grotesque in appearance, lack coordination and finally die during the third or fourth week of postnatal life. Clark, 1934, Anat. Rec. 58: 225–233. Clark, 1935, Proc. Nat. Acad. Sc. 21: 150–152.

hy-2 hydrocephalus-2.—The ventricles of the brain are distended with fluid and the skull enlarged. These brain defects are detectable at least as early as 4 days. Growth is much retarded and there is a high mortality particularly during the first week. Adults are sterile and usually about one half normal size, though the size varies considerably. Zimmermann, 1933, Z. ind. Abst.-u. Vererb. 64: 176–180.

ln leaden.—Phenotypically almost indistinguishable from *d*. Murray, 1933, Am. Nat. 67: 278–283.

my myelencephalic blebs.—Large blisters filled with clear fluid appear on the back of 7–8 mm. embryos and move toward the extremities where they tend to cause bleeding and various foot and eye defects that persist in the adult mouse. The expression of the gene in the adult mouse, at least, appears to be subject to frequent normal overlaps. Bonnevie, 1934, J. Exp. Zool. 67: 443–520.

N naked.—Chromosome 6. Linked with *Ca* (which see). *NN* mice are almost completely hairless from birth; vibrissae absent. Sterile, except that occasional males show low degree of fertility. In *Nn* mice the coat appears almost normal up to 14 days, though usually a little short and dull; thereafter the hairs break off before attaining normal length, so that parts of the skin are naked, other parts covered by hair. Fertility of heterozygotes is normal. David, 1932, Z. Zellforsch. u. mikr. Anat. 14: 616–719.

p pink-eye.—Chromosome 1 (see *C*). Eyes pink, coat much lightened, tending towards brown or yellow. Recognizable at birth by lack of pigment in the eye. Little, 1913, Carnegie Instn. Wash. Pub. No. 179: 11–102.

pa pallid (pink-eye-2).—Chromosome 5 (see *A*). Rather similar phenotypically to *p* but causes more extreme dilution of coat color. Eyes pink, unpigmented. Roberts, 1931, Science 74: 569.

r rodless retina.—Chromosome 4. Probably linked with silver (about 12% crossing-over). Keeler, 1930, Bull. Howe Lab. Ophthalmology 3: 1-11. Rods lacking or reduced in number. Blind. Keeler, 1925, Anat. Rec. 31: 341.

Re rex.—Phenotypically similar to or identical with *Ca*. Crew and Auerbach, 1939, J. Genet. 38: 341–344.

s piebald.—Chromosome 3 (see *hr*). White spotting. There is always a white area on the belly, usually one on the back, often a white blaze on the head, but the amount and location of the spotting is variable, being affected by both modifying genes and environment. In one "all-white" strain 99% of the dorsal area, on the average, is white, but this has been shown to be due to a group of "k" genes capable of causing 3 to 35% dorsal white in the absence of *s*. *Ss* mice may show some white, particularly on the belly. Dunn and Charles, 1937, Genetics 22: 14–42.

Sd short-Danforth.—In heterozygotes the tail is shortened, terminating in a contorted filament, or lacking; sacral region frequently shortened due to malformations of the sacral vertebrae; one or both kidneys may be reduced or missing. Viability reduced. In homozygotes tail is lacking and spinal column shortened, usually terminating at the second lumbar vertebra; anus imperforate; kidneys absent; bladder and urethra sometimes absent. Do not survive more than 24 hours after birth. Dunn, Gluecksohn-Schoenheimer and Bryson, 1940, J. Hered. 31: 343–348.

se short-ear.—Chromosome 2 (see *d*). The ears do not grow after 14 days thus remaining quite short. Prior to 14 days cannot be distinguished from normal sibs. The gene produces several other minor effects, in particular a muscular waviness of the tail that disappears in etherized animals. Lynch, 1921, Am. Nat. 55: 421–426. Snell, 1935, Genetics 20: 545–567.

sh-1 shaker-1.—Chromosome 1 (*see C*). Nervous, rapid, up and down movements of the head. Internal ear histologically normal up to 12 days, thereafter abnormalities appear which are later accompanied by deafness. Recessive, except that *Sh-1sh-1 Vv* mice usually go deaf at from 3 to 6 months of age. Lord and Gates, 1929, Am. Nat. 63: 435–442. Grüneberg, Hallpike and Ledoux, 1940, Proc. Roy. Soc. B 129: 154–173.

sh-2 shaker-2.—Chromosome 7. Linked with *wa-2* (25% crossing-over). Snell and Law, 1939, J. Hered. 30: 447. Nervous movements of the head which are indistinguishable from those of *sh-1sh-1* mice. Clark, 1935, Proc. Nat. Acad. Sc. 21: 247–251.

si silver.—Chromosome 4 (see *r*). Some of the hairs in coat partly or wholly unpigmented. Quite variable. The silvering is more pronounced when one *b* gene or one W^v gene is present. Ordinarily recessive, but partly

dominant in the presence of one W^v gene. Dunn and Thigpen, 1931, J. Hered. 21: 495–498.

st shaker short.—Recognizable at birth by absence or reduction of the tail and by the presence of one or two small blood-blebs in the dorsal median line of the head. Disturbances of equilibrium suggestive of shaker-1 appear at about 5 days. Semicircular canals and endolymphatic appendage are lacking, and the cochlea and cortical organ are abnormal. Deaf. Sterile in both sexes. Bonnevie, 1936, Genetica 18: 105–125.

t type allele of T.

T brachyury.—*Tt* mice are short-tailed (brachyuric). *TT* gives abnormal embryos which die about 11 days after fertilization. Dobrovolskaia-Zavadskaia and Kobozieff, 1934, Arch. zool. exp. et gén. 76: 249–358.

t^0 lethal allele of t.—Tt^0 mice are tailless; t^0t^0 gives abnormal embryos which stop developing at between 5¼ and 7 days embryo age; tt^0 mice are normal. Males heterozygous for t^0 and either *T* or *t* transmit t^0 to more than half their progeny, probably due to an effect of t^0 on segregation. Gluecksohn-Schoenheimer, 1940, Genetics 25: 391–400.

t^1 lethal allele of t.—Tt^1 mice are tailless; t^1t^1 mice die before implantation; t^0t^1 males are sterile, the females normal. Like t^0 in its effect on ratios. Dunn and Gluecksohn-Schoenheimer, 1939, Genetics 24: 587–609.

T^f fused.—Allele of *t*. T^ft mice usually have a kinked tail due to fusion of vertebrae, but there are normal overlaps in some stocks. T^fT^f mice show a more marked expression of the same trait, the tail often being very short. Reed, 1937, Genetics 22: 1–13.

v waltzing.—Shaking movements of the head and a tendency to run in circles. Deaf. Probably due to a defect of the inner ear. Not always distuinguishable phenotypically from *sh-1* and *sh-2*. Gates, 1926, Carnegie Instn. Wash. Pub. No. 337: 83–138.

w type allele of W.

W dominant spotting.—*WW* mice are anaemic, usually living for only a few days after birth. Those surviving long enough to develop a coat are all white with black eyes. In the presence of certain recessive modifying genes, *m*(*w*), at least 3 in number, *W* is partly dominant, *Ww* mice showing 90–98% white. In the absence of the modifiers, *Ww* mice show no spotting; with only some of the modifiers present, the degree of spotting is intermediate. One dose of *s* increases the spotting in *Ww* mice provided some or all of the modifiers are present. A^y tends to reduce the amount of white spotting. Dunn, 1937, Genetics 22: 43–64. Dunn, MacDowell and Lebedeff, 1937, Genetics 22: 307–318.

W^v viable dominant spotting.—Allele of W. W^vw mice are similar to Ww mice. W^vW^v mice usually live to maturity. They are all white with black eyes, usually sterile, but occasionally with a limited fertility. The erythrocyte count is about one half normal. The W^v gene lightens *sisi* and makes *si* partly dominant to *Si*. Little and Cloudman, 1937, Proc. Nat. Acad. Sc. 23: 535–537. Grüneberg, 1939, Genetics 24: 777–810.

wa-1 waved-1.—Hair wavy, vibrissae slightly curly. Recognizable in mice at about 5 days of age because of curling of vibrissae. Quite pronounced at 7 or 8 weeks, thereafter becomes less distinct and in older mice remains only as a slight curling at the tip of the vibrissae and a tendency of the hairs on the back to incline towards the mid-line of the body. Crew, 1933, J. Genet. 27: 95–96.

wa-2 waved-2.—Chromosome 7 (see *sh-2*). Like *wa-1* but more pronounced. Keeler, 1935, J. Hered. 26: 189–191.

Characters Inherited in an Irregular or Undetermined Manner

There are a number of structural and physiological characters in mice which genetic tests have shown to be inherited, but the exact manner of whose inheritance is not yet adequately determined. Most of them give imperfect ratios so that they cannot be classed as simple recessive or dominant factors due to a single gene. These characters are listed and briefly described below and a reference given.

Agglutinin absorption ability of blood corpuscles.—The blood corpuscles of different strains of mice may be classified as strong or weak according to their ability to absorb agglutinin. Strong ability may be inherited as a simple dominant. Gorer, 1936, J. Genet. 32: 17–31.

Anophthalmia.—An anophthalmic strain gives 90% complete eyelessness on both sides and 10% of various degrees of smallness of the eyes. Chase and Chase (in press).

Cleft palate and harelip.—Usually recessive in F_1 but occasionally dominant. Ratios are imperfect, showing variable but usually large number of normal overlaps. Reed, 1936, Genetics 21: 361–374. Steiniger, 1939, Z. Menschliche Vereb. u. Konstitutionslehre 23: 425–462.

Diaphragm imperfectly formed.—Causes death in newborn mice due to leakage of air from ruptures in lungs. Wang, 1938, Anat. Rec. 71: 469–476.

Ectromelie.—Absence of tibia from hind legs. Perhaps recessive with normal overlaps. Rabaud and Hovelacque, 1923, Bull. biol. France et Belgique 57: 401–468.

Eyelids open at birth.—Often not symmetrical on two sides. Perhaps recessive with normal overlaps. Loeffler, 1932, Z. ind. Abst.- u. Vereb. 61: 409–446.

Headdot.—White dot on head. Irregular recessive probably distinct from piebald. Little, 1926, Anat. Rec. 34: 171. Keeler, 1935, Proc. Nat. Acad. Sc. 21: 379–383.

Hound-ear.—Varies from slight reduction of pinna to absence of outer ear. Recessive with numerous normal overlaps. Feldman, 1932, Proc. Sixth Int. Cong. Genet. 2: 51–52. McPheters and Little, 1933, J. Hered. 24: 157–158. Kobozieff and Pomriaskinsky-Kobozieff, 1940, Compt. rend. Soc. biol. 133: 386–389.

Hyperglycaemia and hypoglycaemia.—Grüneberg and Haldane, 1940, Nature 145: 704–705.

Hypotrichosis juvenilis.—The first coat of hair is thin or almost lacking. At 5 weeks the second coat begins to appear and grows in normally, starting at the head and progressing towards the tail. Perhaps due to recessive gene, but the percentage of normal overlaps ranges from 3% to 66% in males, higher in females. Loeffler, 1934, Z. ind. Abst.-u. Vererb. 67: 209–211.

Microphthalmia.—Eyes small or opaque, and show various histological abnormalities. Quite variable. Koch and Gowen, 1939, Arch. Path. 28: 171–176.

Palatal ridges reduced.—One pair of palatal ridges missing. Woolley, 1937, Rec. Genet. Soc. Am. 6: 176–177.

Polydactylism.—Occurs in a small percentage of mice in certain inbred strains. Murray, 1932, Science 75: 312. Fortuyn, 1939, Genetica 21: 97–106.

Posterior duplication.—Varying degrees of duplication of structures at posterior end of body. Lethal in extreme forms. Recessive with normal overlaps. Danforth, 1930, Am. J. Anat. 45. 275–288.

Pseudencephalie.—Brain defect due to failure of the neural groove to close. Perhaps recessive. Bonnevie, 1936, Norske Videnskaps-Akademi I Oslo. I. Mat.-Naturv. Klasse 9: 1–38.

Tail tip pigmentation.—White tail tip, inherited perhaps as recessive with normal overlaps. Grüneberg, 1936, J. Genet. 33: 343–345.

Vaginal occlusion.—Occurred in a number of individuals of silver strain. Marx, 1936, Anat. Rec. 66: 449–454.

INDUCED CHROMOSOME MUTATIONS

X-rays and neutron rays when applied to mature ova and spermatozoa of mice are a prolific source of translocations.* Other sorts of chromosome mutations probably are induced also, but are not detectable by the genetic methods at present available. The detection of translocations is relatively easy, due to the fact that mice heterozygous for a translocation are semi-sterile, consistently producing small litters even when mated to unrelated and entirely normal mice.

T-$F_1$146 translocation-$F_1$146.*—Average size of litters from the mating *T-$F_1$146/+ × +/+ is 4.6 as compared with the normal value for the stock of 8.3. Reduction in litter size is due to death in utero of approximately 45% of the embryos. Most of these embryos die shortly after implantation; a few live to later stages, occasionally even to term, but show brain abnormalities due to failure of the neural groove to close at the anterior end. Of the viable young, one half on the average are semi-sterile, one half normal. The chromosomes involved are 5 and the chromosome carrying *b* (0% crossing over between *a* and break, 20% between break and *b*). Snell, Bodemann and Hollander, 1934, J. Exp. Zool. 67: 93–104. Snell, 1941, Genetics 26: 169.

T-1 translocation-1.—Very little reduction in litter size, but almost 4.6% of the offspring from the cross *T-1*/+ × +/+ show brain abnormalities due to failure of the neural groove to close at the anterior end. These frequently come to term. The evidence that this is a translocation is not complete. Snell and Picken, 1935, J. Genet. 31: 213–235.

RULES FOR ASSIGNING SYMBOLS TO MUTATIONS

The following rules for assigning symbols to mutations have been adopted by the Committee on Mouse Genetics Nomenclature.†

1. The initial letter of the mutant symbol shall be the same as the initial letter of the mutant gene, *e.g.*, *d* for dilution.

2. Additional letters shall be added to the initial letter if necessary to distinguish it from symbols already in use. These shall be, preferably, those immediately following the initial letter, or suggestive letters, espe-

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* Snell, 1935, Genetics 20: 545–567; Snell and Ames, 1939, Am. J. Roent. Rad. Therapy 41: 248–255.

† Dunn, Grüneberg and Snell, 1940, J. Hered. 31: 505–506.

cially consonants, from the rest of the name, e.g., *dw* for dwarf, *ac* for absence of corpus callosum.

3. Recessive mutations shall be indicated by the use of a small initial letter for the symbol of the mutant gene, the type allele being distinguished by a capital letter, e.g., *a* for non-agouti, *A* for agouti.

4. Dominant mutations shall be indicated by the use of a capital initial letter for the symbol of the mutant gene, the type allele being distinguished by a small initial letter, e.g., *Re* for rex, *re* for the type allele of rex.

5. The wild type may also be represented by a + rather than by a letter when this is more convenient, or by a small letter with a + superscript, e.g., + or t^+ for the type allele of *T*, + or a^+ for the type allele of *a*.

6. Multiple alleles (except lethals) shall be indicated by the use of superscripts (always small letters, never capitals) added to the symbol of the original mutant type, e.g., c^e for extreme dilution. It is suggested that the letter selected be the initial letter of the name of the mutation, e.g., T^f for the fused allele of brachyury. The initial letter of the name of the discoverer may also be used. Lethal alleles in a multiple series may be indicated by the use of superscript numerals, e.g., t^0 and t^1 for the lethal alleles of brachyury.

7. Mimics, i.e., mutants of similar phenotype but different location, shall be indicated either by entirely different names and symbols (e.g., *ln* for leaden and *d* for dilution) or by the same name and symbol with the addition of distinguishing numbers (e.g., *wa-1* for waved-1 and *wa-2* for waved-2). The latter procedure is not recommended.

8. In published articles in American journals in which symbols are used, the symbols should be set in italics.

The Chromosomes of the Mouse

The mouse has 20 pairs of chromosomes. In males, one pair consists of two chromosomes of unequal size. These are the sex chromosomes, the X and the Y, the X being the larger.*

Presumably, in course of time, the number of linkage groups in the mouse will come to equal the number of chromosome pairs. At the present time seven linkage groups are known, *p c sh-1*, *d se*, *hr s*, *r si*, *a pa*, *Ca N* and *sh-2 wa-2*. In addition to these, nine genes, *b*, *dw*, *f*, *hy-1*, *ln*, *T*, *v*, *W* and *wa-1* have been tested against most of the other known genes without

* Painter, 1928, Genetics 13: 180–189.

showing linkage, so that most or perhaps all of these mark additional chromosomes, making perhaps sixteen chromosomes in all with known marker genes.

No known mutant gene in the mouse, or in fact in any of the rodents, sufficiently clear cut in its effects to serve as a "marker" gene, is sex-linked. In view of the large size of the X chromosome and the ease with which sex-linked mutations, if they occur, can be detected, this is a noteworthy fact.

In all cases of linkage sufficiently well tested to give critical evidence, the crossover percentage has been higher in the female than in the male. In accord with this, the number of chiasmata observed during gametogenesis is higher in the female than in the male.*

Negative Linkage Data

Most of the gene mutations that have been found in mice have been tested for linkage against other known gene mutations. Where these tests have led to the discovery of a linkage, this is indicated in the section on Gene Mutations (p. 234). In the majority of cases no linkage has been found. These negative linkage data are summarized in the accompanying table. In this table all the genes are listed in the first vertical and also in the first horizontal line. Where several genes lie on the same chromosome they are listed as a unit. The crossover data for any two genes are found in the rectangle where the horizontal line from one gene and the vertical line from the other gene intersect. In a number of cases several tests have been made for a single pair of genes. In such cases the data from one test only, that involving the most animals or for other reasons the most satisfactory, have been use. The data given at each intersection consist of the following:

1. A number referring to a reference in the bibliography.

2. An abbreviation indicating the type of cross used. The abbreviations are:

BC, a cross of the type $AaBb \times aabb$

F_2, a cross of the type $AaBb \times AaBb$

MC, a cross of the type $AaBb \times Aabb$

3. The observed crossover per cent plus or minus its standard error, or where this cannot be given, the data themselves. In the case of backcross (BC) data, the standard error has been calculated from the tables given by

* Bryden, 1933, J. Genetics 27: 421–433.

TABLE OF NEGATIVE LINKAGE DATA

	d se	*hr s*	*r si*	*a pa*
c p sh-1	9BC 50.0 ± 2.7	8BC 47.8 ± 1.4	12BC 51.1 ± 3.2	8BC 49.3 ± 1.6
d se		9BC 46.4 ± 1.3	12BC 51.3 ± 5.5	13F_2 48.9 ± 2.2
hr s			12BC 41.5 ± 9.3	8BC 46.2 ± 1.7
r si				12BC 52.8 ± 5.5

TABLE OF NEGATIVE LINKAGE DATA.—(*Continued*)

	N Ca	*sh-2 wa-2*	*b*	*dw*
c p sh-1	16BC 53.6 ± 3.5	10F_2 47.1 ± 2.7	7BC 49.7 ± 0.8	17F_2 > 57.3 ± ?*
d se	16BC 49.3 ± 4.6	10F_2 54.6 ± 2.4	9BC 45.5 ± 1.3	17F_2 > 57.3 ± ?*
hr s	16BC 60.3 ± 6.1	4F_2 57.1 ± 6.0	9BC 51.4 ± 1.3	17MC 14:17:6:*4*
r si	16BC 61.5 ± 8.4	4F_2 44.7 ± 9.5	12BC 49.2 ± 6.5	
a pa	16BC 54.3 ± 2.6	4F_2 56.3 ± 7.3	7BC 50.3 ± 0.8	17MC 35:39:*10*:15
N Ca		4BC 53.2 ± 4.8	16BC 53.5 ± 2.8	17MC 26:33:*10*:11
sh-2 wa-2			4F_2 50.5 ± 6.9	4F_2 48.2 ± 8.1

* Crossover per cents greater than 57.3 are not given in Stevens' tables.

TABLE OF NEGATIVE LINKAGE DATA.—(*Continued*)

	f	*hy-1*	*ln*	*Re*
c p sh-1	3F_2 51.4 ± 5.2	5F_2 54.3 ± 6.0	14F_2 182:61:96‖	6BC 51.8 ± 5.5
d se	3F_2 45.3 ± 4.3	5F_2 >57.3 ± ?*	14BC 51.7 ± 3.5	6BC 50.0 ± 6.4
hr	3F_2 44.9 ± 7.6	5F_2 46.6 ± 7.2	14BC 42.8 ± 5.2	6BC 32:22:*13*:11¶
r si	3F_2 R:r:27:6†	5F_2 R:r::23:5‡		
a pa	3F_2 55.5 ± 6.9	5MC 46:37:12:*11*	14BC 50.0 ± 4.4	6BC 47.1 ± 8.6
N Ca	3BC 48.6 ± 5.9	5MC 31:42:*8*:13	14BC 46.8 ± 3.9	
sh-2 wa-2	3F_2 >57.3 ± ?*	5F_2 35.3 ± 5.5§	4F_2 51.4 ± 7.5	
ac	No linkage tests have been made			
b	3F_2 56.1 ± 3.5	5MC 14:14:3:*4*	14BC 50.4 ± 2.9	6BC 60.5 ± 7.1
dw	3F_2 56.4 ± 7.1	5F_2 >57.3 ± ?*	14F_2 48.3 ± 8.4	
f		5F_2 56.0 ± 7.4	3BC 50.0 ± 4.1	
gl	No linkage tests have been made			
hy-1			5F_2 53.9 ± 6.9	
hy-2	No linkage tests have been made			
ln				6BC 22:32:*9*:15

* Crossover per cents greater than 57.3 are not given in Stevens' tables.

† Only the 33 flexed mice were classified as to whether they were type or rodless.

‡ Only the 28 hydrocephalus-1 mice were classified as to whether they were type or rodless.

§ Tests of 14 of the *sh-2sh-2 Hy-1* mice for the presence of *hy-1* showed more than the expected number of crossovers in this group.

‖ The original cross was *c* × *ln;* the expected F_2 ratio is 190:63:85.

¶ Waved-1 as well as rex was involved in this backcross, giving an expected ratio of 3:3:1:1.

TABLE OF NEGATIVE LINKAGE DATA.—(*Continued*)

	T	*v*	*W*	*wa-1*
c p sh-1	2BC 48.1 ± 5.7	9BC 50.3 ± 2.6	18BC 52.4 ± 2.9	11BC 55.5 ± 3.7
d se	2BC 49.2 ± 4.5	9BC 47.0 ± 1.3		11BC 52.1 ± 3.6
hr s	2BC 43.7 ± 5.9	9BC 48.4 ± 1.3	8BC 48.6 ± 2.2	1F_2 55.4 ± 5.6
r si	2BC 45.5 ± 5.0		12BC 41.9 ± 7.6	1F_2 43.7 ± 7.4
a pa	2BC 47.0 ± 5.0	9BC 55.8 ± 4.7	18BC 51.3 ± 2.6	11BC 51.1 ± 2.5
N Ca	2BC 51.0 ± 5.0	16BC 60.0 ± 9.1	16BC 47.0 ± 7.0	1MC 18:24:*11*:7
sh-2 wa-2	2BC 53.4 ± 5.3	4F_2 94:54†	10BC 52.9 ± 6.1	1BC 52.8 ± 5.9
ac	No linkage tests have been made			
b	2BC 45.5 ± 4.8	9BC 49.9 ± 1.3	17BC 51.7 ± 6.6	11BC 48.1 ± 2.5
dw	2MC 15:23:*3*:7	17MC 26:12:*7*:6	17MC 12:17:*4*:7	1F_2 >57.3 ± ?*
f	2BC 46.8 ± 3.4	3F_2 >57.3 ± ?*	3MC 28:23:*6*:9	1F_2 53.6 ± 5.6
gl	No linkage tests have been made			
hy-1	5MC 48:48:*12*:18	5F_2 42.0 ± 9.4	5MC 21:20:*5*:4	
hy-2	No linkage tests have been made			
ln	2BC 50.5 ± 4.8	14F_2 45.9 ± 6.5	14BC 48.7 ± 3.3	1F_2 >57.3 ± ?*
my	No linkage tests have been made			
Re		6BC 34:20:*14*:10‡		
st	No linkage tests have been made			
Sd	No linkage tests have been made			
T		2BC 46.5 ± 5.4	2BC 49.3 ± 5.9	1BC 47.0 ± 3.4
v				1F_2 50.0 ± 7.0
W				1BC 56.7 ± 6.4

* Crossover per cents greater than 57.3 are not given in Stevens' tables.

† The waltzing and shaker-2 animals were grouped into one class; the expected ratio is 83:65.

‡ Waved-1 as well as rex was involved in this backcross, giving an expected ratio of 3:3:1:1.

Castle.* In the case of F_2 data, the crossover per cent and the standard error have been calculated from the tables given by Stevens.† In the case of all data from mixed crosses (MC) the data are given in full. The data are given in such order that the expected ratio is *AB*:*Ab*:*aB*:*ab*::3:3:1:1, and of the last two numbers, the one that represents the crossover class is given in italics.

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Chapter 6

THE GENETICS OF SPONTANEOUS TUMOR FORMATION

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INTRODUCTION

The application of genetic methods in the analysis of the incidence of spontaneous tumors in mice is a matter of considerable complexity. Various factors and influences serve to modify the actual effects of genes. Yet in spite of this fact there is compelling evidence that the genetic constitution of an organism plays a part in determining whether or not it will develop a tumor or tumors.

The earliest work which contributed to this conclusion was that of Tyzzer (101), J. A. Murray (73), Bashford (8), Haaland (43) and Loeb (48). By 1912 these investigators had independently demonstrated that families and strains of mice differed in their capacity for producing breast tumors.

A good deal of this earlier work lacked certain qualities which would have greatly increased its accuracy. In some cases histological diagnosis of the palpated nodules was absent. In others the number of individuals studied was none too large. In some, knowledge of the more remote

ancestry of the material was fragmentary and insufficient. *In all, the long continued process of inbreeding so necessary to establish the genetic homogeneity of the strains before they were used was lacking.*

This handicap weighed heavily against the later work of Slye (86–92) and of Lathrop and Loeb (48–50). It has provided the most important point of difference between the work completed before 1925 and that of the fifteen years that have followed.

It is not at all surprising that the earlier work lacked this preliminary process of genetic purification. There was more than one reason why this was the case.

In the first place extra-peritoneal tumor nodules in mice are striking and superficially obvious. To the early workers in experimental genetics they gave a false sense of simplicity and a feeling that a tumor was something as definite and predictable as coat color or any of the routine Mendelian characters.

Then too, the great interest of all in the cancer problem was a constant challenge to begin work on it without delay. The period of years necessary in order to carry out the required preliminary inbreeding was not at all appealing to the geneticists who had found abundant "surface gold" in the shape of genetic differences capable of immediate analysis. Once an investigator had embarked upon a program of rapid genetic methods, it was not likely that he would scrap that work, retrace his steps and make a fresh start with more uniform material.

Yet it is probable that more progress would have been made had this been done. The experiments of Tyzzer, of J. A. Murray and of Haaland reached the limit of their potentiality for detailed analysis by showing that female mice with breast tumors had more female ancestors with similar tumors than did tumor-free animals. The findings of Loeb and Lathrop indicated that, in addition, there were "strain differences" in the age at which such tumors developed, and that the actual incidence of the tumors might have a quantitative basis on multiple factors. Not even the later painstaking statistical analysis of their data by Bernstein (9) could add basic accuracy or further knowledge of the nature of the genetic process.

The most extensive series of experiments between 1900 and 1930 were those of Slye who raised and observed thousands of animals. These represented many *pedigree* lines of descent but had to rely by their very nature on the *ex post facto* combination and summation of a large number of scattered small-progeny matings in order to establish trends, groups or genetic principles.

In the meantime Wright (105, 106), who had started the genetic analysis of a large number of strains of closely inbred guinea-pigs at the U.S. Department of Agriculture, began to publish results which showed, (*1*) that the incidence of certain morphological genetic abnormalities could differ in different inbred lines, and (*2*) that non-genetic factors often influenced the incidence of such characters, within a strain, more than did genes. The foundation for a much more complex interpretation of the bio-genetics of tumor formation was thus laid by evidence of a far from simple situation in the genetics of other growth abnormalities.

The history of the development of our knowledge concerning the genetics of spontaneous tumor formation in mice has followed the trend of recognizing more and more complicating factors.

Slye's original theory (1913–1937) that all types of cancer in mice were due to a single recessive Mendelian gene has been replaced by evidence that there is a high degree of specificity as regards type and location of neoplastic change. Various physiological factors such as age, sex and coat color have some influence on the expression of the genetic constitution and its relation to tumor formation.

Lynch (61) gave evidence suggestive of the possible partial dominance of the tendency to form breast tumors. Little (52) showed that Slye's data were not incompatible to some such interpretation. The discovery of an extra-chromosomal maternal influence on the incidence of breast tumors in mice was announced by the staff of the Roscoe B. Jackson Memorial Laboratory (44) and independently by Korteweg (46). This was further investigated by Murray and Little (77). Bittner (15) made an important discovery that an extra-chromosomal influence affecting breast tumor incidence could be transmitted from parent to offspring apparently through the milk.

In the meantime data were being gathered to show that lung tumors (chiefly adenocarcinomas) and non-epithelial tumors, chiefly lymphosarcomas, fibrosarcomas and endotheliomas, were two other categories of neoplasms quite largely distinct from mammary carcinomas and from one another. A fourth group, strictly speaking a subdivision within the non-epithelial tumor class, may well be made to include at least certain of the leukemias. The excellent work of MacDowell, Richter and others (67–70) supports such a subdivision. All of these steps were clear indicators of an increasing complexity in the inherent nature of the genetic process.

We may very briefly review the more important data which have led to the creation of at least four distinct biological groups of spontaneous tumors in mice.

Mammary Epithelial Tumors

Evidence that the tendency to form such tumors is not due to the action of a single recessive gene.—Preliminary evidence was provided by the work of Lynch (61) who, in a series of crosses between various strains of mice, showed that mammary tumors occurred in F_1 animals. The strains used were the best then available but left much to be desired as regards the extent of genetic analysis previous to crossing.

Statistical analysis based on tabulations of Slye's extensive pedigree data by Little (52) showed that her results could be as well explained on the basis of dominance of the tendency to form breast tumors as on its recessive nature.

Data published by the Staff of the Roscoe B. Jackson Memorial Laboratory (44) and confirmed independently by Korteweg (46) who published at almost the same time showed,

1. That F_1 hybrids between "high" breast tumor and "low" breast tumor strains formed large numbers of mammary tumors.

2. That this result was more in agreement with a theory of dominance than of recessive nature of the tendency to form these tumors.

Evidence of an extra-chromosomal influence.—These same experiments showed that a most interesting and unexpected difference exists between the reciprocal crosses which produced such F_1 generation mice.

Where the cross was made between "high tumor" strain female and "low tumor" strain male, the rate of breast tumor incidence in F_1 generation females approached that of the "high tumor" parent strain. When, however, the cross was made between "low tumor" strain females and "high tumor" strain males, the tumor rate in F_1 females was little if any above that of the "low tumor" parent strain. The F_2 generations followed closely the rate of mammary tumor incidence shown by the type of F_1 generation from which they were derived. These results have now been confirmed and established by the work of a number of investigators.

Tables taken from Murray and Little (77) show the incidence and age distribution of mammary tumors in the F_1 hybrids from reciprocal crosses between the "high tumor" dba strain and the "low tumor" C57 black strain. In Table 1 is the hybrid generation derived from the cross dba ♀ × C57 black ♂.

It will be noted that the rate of tumor incidence usually falls between 30 and 45 per cent. This may be contrasted with the reciprocal generation (Table 2) produced by crossing C57 black females with dba males.

Here the tumor incidence averages approximately 6 per cent. The Difference is mathematically significant.

Table 1
dB F_1 Generation (dba ♀ × C57 Black ♂)

Age Group	Deaths		No. Alive at Start of Period	No. Which Later Formed Tumors	Percentage Which Formed Tumors
	Tumor	Non-tumor			
151–180	1	5	113	45	39.82
181–210	0	5	107	44	41.12
211–240	1	3	102	44	43.13
241–270	0	3	98	43	43.87
271–300	0	1	95	43	45.26
301–330	1	0	94	43	45.74
331–360	0	1	93	42	45.16
361–390	1	0	92	42	45.65
391–420	1	2	91	41	45.05
421–450	5	1	88	40	45.45
451–480	5	1	82	35	42.68
481–510	4	2	76	30	39.47
511–540	5	3	70	26	37.14
541–570	0	5	62	21	33.87
571–600	3	4	57	21	36.84
601–630	2	3	50	18	36.00
631–660	2	1	45	16	35.55
661–690	2	4	42	14	33.33
691–720	1	2	36	12	33.33
721–750	2	1	33	11	33.33
751–780	4	4	30	9	30.00
781–810	1	3	22	5	22.72
811–840	0	4	18	4	22.22
841–870	0	1	14	4	28.57
871–900	2	3	13	4	30.76
901–930	0	2	8	2	25.00
931–960	2	2	6	2	33.00
961–990	0	2	2	0	

The tumor incidence in the reciprocal F_2 generations shows that the difference still persists. In Table 3 are included the F_2 mice descended from

Table 2
Bd F_1 Generation (C57 Black ♀ × dba ♂)

Age Group	Deaths		No. Alive at Start of Period	No. Which Later Formed Tumors	Percentage Which Formed Tumors
	Tumor	Non-tumor			
151– 180	0	1	379	23	6.06
181– 210	0	0	378	23	6.08
211– 240	0	3	378	23	6.08
241– 270	0	1	375	23	6.13
271– 300	0	9	374	23	6.14
301– 330	0	8	365	23	6.30
331– 360	1	13	357	23	6.44
361– 390	1	20	343	22	6.41
391– 420	1	30	322	21	6.52
421– 450	0	28	291	20	6.87
451– 480	0	29	263	20	7.60
481– 510	0	18	234	20	8.54
511– 540	2	8	216	20	9.25
541– 570	0	3	206	18	8.73
571– 600	1	9	203	18	8.86
601– 630	1	9	193	17	8.80
631– 660	3	13	183	16	8.74
661– 690	0	13	167	13	7.78
691– 720	2	10	154	13	8.44
721– 750	1	16	142	11	7.74
751– 780	1	10	125	10	8.00
781– 810	1	11	114	9	7.89
811– 840	2	13	102	8	7.84
841– 870	1	12	87	6	6.89
871– 900	1	11	74	5	6.75
901– 930	1	6	62	4	6.45
931– 960	1	15	55	3	5.45
961– 990	1	10	39	2	5.12
991–1020	0	4	28	1	3.57
1021–1050	0	5	24	1	4.16
1051–1080	0	4	19	1	5.26
1081–1110	0	11	15	1	6.66
1111–1140	1	2	4	1	25.00
1141–1170	0	0	1	0	
1171–1200	0	1	1	0	

inbred dBF_1 animals (F_2 from the cross dba ♀ × C57 black ♂). This is the dBF_2 generation.

Table 3
dB F_2 GENERATION

Age Group	Deaths		No. Alive at Start of Period	No. Which Later Formed Tumors	Percentage Which Formed Tumors
	Tumor	Non-tumor			
211–240	1	0	664	236	35.54
241–270	1	1	663	235	35.44
271–300	1	4	661	234	35.40
301–330	10	52	656	233	35.51
331–360	13	49	594	223	37.54
361–390	15	53	532	210	39.47
391–420	17	34	464	195	42.02
421–450	16	16	413	178	43.09
451–480	9	10	381	162	42.51
481–510	15	6	362	153	42.26
511–540	17	7	341	138	40.46
541–570	13	8	317	121	38.17
571–600	13	15	296	108	36.48
601–630	14	20	268	95	35.44
631–660	11	16	234	81	34.61
661–690	15	10	207	70	33.81
691–720	8	11	182	55	30.21
721–750	7	15	163	47	28.83
751–780	10	19	141	40	28.36
781–810	5	9	112	30	26.78
811–840	6	12	98	25	25.51
841–870	6	7	80	19	23.75
871–900	4	13	67	13	19.40
901–930	3	12	50	9	18.00
931–960	3	14	35	6	17.14
961–990	3	8	18	3	16.66
991–1020	0	7	7	0	

The incidence in all animals which lived as long as or beyond the age of the animal in which a tumor first appeared is 35.54 per cent.

Table 4
Bd F_2 GENERATION

Age Group	Deaths		No. Alive at Start of Period	No. Which Later Formed Tumors	Percentage Which Formed Tumors
	Tumor	Non-tumor			
211– 240	0	2	687	41	5.96
241– 270	0	1	685	41	5.98
271– 300	0	5	684	41	5.99
301– 330	0	13	679	41	6.03
331– 360	3	28	666	41	6.15
361– 390	3	31	635	38	5.98
391– 420	3	35	601	35	5.82
421– 450	2	45	563	32	5.68
451– 480	0	28	516	30	5.81
481– 510	4	11	488	30	6.14
511– 540	2	13	473	26	5.49
541– 570	1	23	458	24	5.24
571– 600	0	16	434	23	5.29
601– 630	3	20	418	23	5.50
631– 660	0	30	395	20	5.06
661– 690	4	26	365	20	5.47
691– 720	0	23	335	16	4.77
721– 750	3	22	312	16	5.12
751– 780	2	14	287	13	4.52
781– 810	4	27	271	11	4.05
811– 840	1	36	240	7	2.91
841– 870	0	34	203	6	2.95
871– 900	2	31	169	6	3.55
901– 930	4	37	136	4	2.94
931– 960	0	41	95		0.00
961– 990	0	28	54		0.00
991–1020	0	17	26		0.00
1021–1050	0	5	9		0.00
1051–1080	0	3	4		0.00
1081–1110	0	1	1		0.00

In sharp contrast to this is the low incidence (5.96 per cent) among the BdF_2 mice produced by inbreeding the BdF_1 animals (Table 4).

The eventual weakening and disappearance of the high tumor producing tendency has been shown by Murray and Little (80) (Table 5) in a series of backcross generations which were intended to provide a test of the relative importance of genes and of other influences.

The data obtained from first generation animals backcrossed with parent strains showed only a slight decrease in the incidence of mammary tumors in those animals with extra-chromosomal influences (E) derived from "high" tumor female ancestors. These backcross generations are shown in the

Table 5
INCIDENCE OF MAMMARY CANCER

Stock	dba	dBF_1	dBF_2	BdF_1	BdF_2	A	B	C	D
Number	297	113	664	379	687	250	252	250	244
Observed tumors	151	45	236	23	41	6	90	1	83
Formula	CCCCE	CCccE	CCccE	CCcce	CCcce	CCCce	CCCcE	Ccccc	CcccE
Per cent of cancer	50.84	39.82	35.54	6.06	5.96	2.40	35.71	0.41	34.00

columns marked B and D in Table 5. The animals descended from "low" cancer females with extra-chromosomal influences (e) showed a greater proportional decrease in incidence of mammary tumors (columns A and C, Table 5). The relative independence of all from chromosomal influences (C = high tumor, c = low tumor) is also shown in this table for A and B had three representatives of C while C and D had only one.

The system of matings used, in further studies of advanced backcross generations, to concentrate the chromosomes from respective parent strains is shown in Table 6.

Starting with individuals of the 8th backcross generations (Table 6), which were virtually homozygous, a variety of crosses were made. Individuals from these crosses were identified as follows: S, T, U and V were animals originally derived from maternal ancestors with the "high" tumor extra-chromosomal influence (E). They were, however, eight generations removed from the pure strain originally employed. Had the extra-chromosomal influence remained unchanged, there should have been approximately 196 mammary tumors among the 372 mice recorded. Actually there were 6. This is only 3 per cent of the former tumor rate. The extra-

chromosomal influence has, therefore, largely disappeared. When crosses W, X, Y and Z which lacked the extra-chromosomal high tumor influence are compared with these, there are found to be 4 tumors when 122 were expected. The percentage is similar to the previous crosses but the original extra-chromosomal influence was different.

Having eliminated the extra-chromosomal influence, we may next compare the various crosses as regards their chromosomal composition. Crosses S and U should resemble the original low cancer strain. Actually this was the case as no mammary tumors were recorded in them.

Table 6

Per Cent C57 Black Chromatin	Female Male	Female Male	Per Cent dba Chromatin
50	dBF_1 × Blk	BdF_1 × dba	50
75	1st BC × Blk	1st BC × dba	75
87.5	2nd BC × Blk	2nd BC × dba	87.5
93.7	3rd BC × Blk	3rd BC × dba	93.7
96.9	4th BC × Blk	4th BC × dba	96.9
98.4	5th BC × Blk	5th BC × dba	98.4
99.2	6th BC × Blk	6th BC × dba	99.2
99.6	7th BC × Blk	7th BC × dba	99.6
99.8	8th BC × Blk	8th BC × dba	99.8

Crosses T, V, W and Y had roughly the same formulae as the original outcross BdF_1 and BdF_2. The number of animals observed should have given a total of 24 mammary tumors if the tendency to form mammary tumors had been transmitted through the chromosomes. Actually 7 or 28% of that number were observed.

Crosses X and Z should be comparable to virgin females of the "high tumor" strain. There should have been 144 mammary tumors. Actually there were 3. Even if the extra-chromosomal influence was ruled out, there should have been approximately 14 mammary tumors formed.

There has, therefore, been a very clear decrease in cancer incidence which requires further study.

Evidence that at least part of the extra-chromosomal influence can be transferred by foster nursing.—There are two important ways in which evidence of the importance of foster nursing in determining the incidence of mammary tumors in mice can be obtained.

The first is by the direct transfer of new-born young, reported and extensively studied by Bittner (13, 18, 19, 22–28, see also Chapter 9). This method brought to light the entirely unexpected and very interesting fact that the new born young from "high" breast tumor stocks, when transferred to nursing females of a "low" breast tumor stock, develop into animals which in later life show an incidence of breast tumors very similar to that of their foster mother. To a considerable degree the converse is also true. The incidence of breast tumors among mice of "low" tumor strains can be materially increased if they are fostered by "high" tumor females (Table 7).

Table 7

Stock	Incidence of Breast Cancer
"High" stock females (unfostered)	83.6%
"High" stock females (fostered)	7.9%
"Low" stock females (unfostered)	0.5%
"Low" stock females (fostered)	approximately 9.0%
F_1 ♀'s produced by H ♀ × L ♂ (unfostered)	94.9%
Similar mice fostered by H ♀	95.0%
Similar mice fostered by L ♀	0.0%
F_1 ♀'s produced by L ♀ × H ♂ (unfostered)	1.9%
Similar mice fostered by H ♀	93.0%
Similar mice fostered by L ♀	8.0%

The second method of testing the influence of foster mothers is by the transfer of fertilized ova from the fallopian tubes of "high" breast tumor mice to the uteri of pregnant "low" tumor females. This technique as used by Fekete and Little has given rise to a considerable number of viable young developed from transferred ova. These mice upon maturity have shown a breast tumor incidence characteristic of the strain from which their foster mother was derived. The exact quantitative relationship as regards breast tumor incidence among the fostered and transferred mice obtained by the two methods has not yet been determined. The possibility remains that the intra-uterine influences may prove to be more extensive and stronger than those of the milk alone. On the other hand no such difference may be obtained. The matter is under investigation.

Evidence that genetic factors also influence the incidence of breast tumors.—Perhaps the most interesting experiments to test this point are those recently conducted by Bittner (25, 26). These are based upon the fact that reciprocal crosses between "high" (A) and "low" (C57 black) breast tumor strains produce very different degrees of tumor incidence in F_1. These were compared with the pure "high" and "low" tumor stocks by fostering young in both series.

The results are summarized in Table 7.

According to Bittner's theory all F_1 hybrids whether produced from H ♀ × L ♂ or from L ♀ × H ♂ should carry one group of "high" tumor genes from their high parent. When to this genetic tendency the extra-chromosomal influence is added, the results are very different from the parallel fostering between the pure stocks, one of which lacks the "high" tumor genes. Thus pure "low" stock females fostered by "high" stock produce only 9% breast tumors, while F_1 females, themselves "low," produce 93% breast tumors when fostered by high tumor females.

While further experiments are necessary, the evidence at present favors Bittner's theory that some influence of genes is active.

Evidence that hormonal influences also affect the incidence of breast tumors.—Primary evidence for this theory is to be found in the comparative behavior of breeding and virgin females in three high tumor strains of mice. These strains are designated respectively as C_3H, dba and A. The approximate incidence of breast tumors is shown in Table 8.

Table 8

Stock	Virgin ♀	Breeding ♀
C_3H	95%	93%
dba	51%	85%
A	5%	84%

It is very evident that the absence of pregnancy and lactation has a markedly different effect in the three strains.

Further evidence of hormonal influence has been derived from the experiments of Bagg and others (5–7) who have shown that forced breeding without opportunity for nursing increases the incidence of breast cancer in animals where some genetic tendency to form such cancer exists.

There is also a series of experiments involving the artificial prolongation of lactation and nursing in mice reported by Fekete (40). In this case the

incidence of breast cancer was somewhat reduced as compared with normal breeding females.

All of this suggests that the cyclic changes to which the breast tissues are subjected in pregnancy and lactation are periods during which the risk of setting up a neoplastic process is very definitely increased.

Evidence that coat color may play a part in influencing the incidence of mammary tumors.—It will be well at the outset to make clear the fact that various degrees of incidence of breast tumors exist in distinct inbred strains of different coat colors. This, however, does not necessarily mean that coat color *per se* affects the incidence directly or even that it represents a general physiological type which is more or less susceptible. Selection which establishes any characteristic coat color for a given inbred strain may also fix the

Table 9

Generation	Total Yellow	Total Non-yellow	Per Cent of Yellow Mice with Breast Tumors	Per Cent of Non-yellow Mice with Breast Tumors
F_1	57	54	38.6	64.8
F_2	156	223	37.2	51.6
Total	213	277	37.5	54.2

tumor incidence coincident with but entirely independent of the question of color.

The real test of the influence of coat color depends upon the comparison of different colored animals within the same generation of mice, where other genetic influences have been equalized as nearly as possible.

The opportunity to study this type of situation is offered by comparing yellow and non-yellow mice among the animals of F_1 and F_2 generations following an outcross.

Since all yellows so far observed are heterozygous, being A^ya or A^yA in formula, the F_1 generation of a cross with *aa* (non-yellow) mice consists of yellows and non-yellows in approximately equal numbers.

The F_2 generation gives yellows and non-yellows in proportions which vary according to the color of the F_1 animals selected for breeding.

In a cross reported by Little (54) the figures shown in Table 9 were obtained.

This is, in all probability, a significant difference and is, therefore, of interest.

In analyzing the possible reason for the decreased incidence of mammary tumors in yellow mice, the following suggestions were made by the writer:

"A study of the physiology of reproduction of yellow and non-yellow mice within the yellow stock suggests that the yellows pass through their reproductive cycle earlier than do the non-yellows. The *duration* of the cycle in the two forms is essentially equal. This fact would satisfactorily explain the earlier incidence of mammary tumors in yellow mice.

"The lower incidence of mammary tumors in yellows as compared with non-yellows may be at least in part due to the same phenomenon. This would follow because the opportunity for mammary tissue in yellow mice of cancer age to be continuously affected by ovarian secretion would be less than in non-yellows. This would result in a higher percentage of yellows reaching an age at which stimuli from the ovary ceased before the mammary tissue had reached an age at which tumor formation is most frequent."

These data show that it is not accurate to lump together different colored mice in calculating the incidence of mammary tumors. They also indicate the need for further study of this general topic.

Summary.—To summarize the situation as regards epithelial breast tumors in mice, one may state:

1. These are the commonest type of spontaneous neoplasm in unselected material.

2. Strains have been produced by inbreeding and selection which may give as high as 93% or as low as 0.5% incidence of these tumors in breeding females.

3. There is conclusive evidence that the incidence of these tumors is not due to a single recessive gene.

4. There is a definite extra-chromosomal influence which is directly transferable from female parent to her progeny.

5. This influence is at least in part, if not entirely, transferable through the milk of the mother.

6. It is probable that genetic factors also play a part in determining the tendency to form tumors.

7. Hormonal influences also affect the incidence of breast tumors.

Epithelial Lung Tumors

Most of the observed lung tumors in mice are epithelial in origin, being of the adenoma, adenocarcinoma or carcinoma simplex types. These

tumors in their incidence and relationship to various genetic factors present interesting contrasts with the mammary group. We may review briefly certain of these differences.

Absence of extra-chromosomal influence.—It will be remembered that reciprocal crosses between strains that were "high" and those that were "low" in breast tumors gave very different results. Such is not the case in similar crosses between "high" and "low" lung tumor strains.

Lynch (63, 64) gave the first data, describing in a preliminary way crosses of this sort between two inbred strains. She mentioned no difference between reciprocal crosses but also gave no figures to differentiate between them. This was not surprising, for, at that period, no such distinction between reciprocal crosses had been described for mammary tumors where later it was found to exist. A later paper by the same writer (65) gave further results of a similar nature.

The most conclusive data on this point, however, are those of Bittner (23) who, having corrected his figures by the elimination of the disturbing factor of "breast tumor" incidence, found the results shown in Table 10.

Table 10

Cross	F_1 Generation		F_2 Generation	
	No. Mice	Per Cent Lung Tumor	No. Mice	Per Cent Lung Tumor
High ♀ × Low ♂	203	76.4	204	59.3
Low ♀ × High ♂	202	76.7	222	54.1

There is no evidence, therefore, of "extra-chromosomal" influence.

"Dominant" nature.—Lynch (63) gave as one of her conclusions the statement that the tendency to form lung tumors in mice appeared to be "dominant" in heredity.

She, however, quite properly recognized the fact that dominance was far from being regular or complete.

Again, her later work as well as that of Andervont and Bittner (1, 13) provided further supporting evidence.

It remained for Bittner (26), however, to give the most complete data on this question. Using, from his data, comparable groups of mice in different generations, we find the results shown in Table 11.

Table 11

	No.	Per Cent Lung
Parent stock C57 black virgin ♀ ♀	133	0.0
Parent stock A virgin ♀ ♀	221	90.0
F_1 hybrids virgin ♀ ♀	367	87.5
F_2 hybrids virgin ♀ ♀	376	67.3

The close similarity of the lung tumor incidence in the high tumor stock and in the F_1 hybrids is striking.

There is evidently a small percentage of potentially and genetically lung tumor animals which fail to develop the neoplasms to a degree or at a sufficiently early age to be recorded. This percentage may be taken as 11.3 which represents an average of 10.0 and 12.5, the A stock and F_1 percentage respectively of normal overlaps.

Using 88.7% as the incidence of lung tumors in a stock in which all animals carried the hypothetical dominant gene for these neoplasms, we may calculate the expectation for F_2 as 75% of that figure or 66.5. The actual percentage observed in that generation was 67.3. The close degree of correspondence between the calculated and observed figures is strong evidence in support of the theory that a dominant Mendelian gene may, in certain crosses, play the main role in determining the incidence of these tumors.

The situation is not, however, quite so simple. Certain crosses of other stocks recently made by Heston (unfinished data) and reported at the 1940 meeting of the American Association for Cancer Research show that modifying genes or other genetic agents influence the percentage of lung tumors formed. Not all "low" tumor stocks behave in a similar manner when crossed with a single "high" tumor strain.

Furthermore, the percentage of mice showing multiple nodules in the lungs was quite different when certain "low" tumor strains were employed from what it was when others were used.

The age at which the nodules became visible also varied according to the parent strains used.

We can thus conclude that the available evidence suggests that a dominant gene is at times clearly indicated but that its influence is subject to modification by secondary genes which affect actual incidence of *any* lung nodule, the *number* of nodules, and the age at which they are usually formed.

Hormonal influences.—Although the distribution of lung tumors is not exactly equal between the sexes, the investigations reported by various workers give conflicting results.

Slye, Holmes and Wells (93) recorded 57.4% of their lung tumors in female mice and 42.6% in males. Lynch, on the other hand, in a large group, obtained among females an incidence of 16% and in males 22%.

Bittner's figures are again the most extensive available. The incidences in the two sexes and in various generations are shown in Table 12.

Table 12
LUNG TUMOR INCIDENCE FOLLOWING RECIPROCAL CROSSES BETWEEN A AND C57 BLACK (B) STRAINS

Generation	No.	Per Cent Tumor	Generation	No.	Per Cent Tumor
ABF_1 ♂♂	91	92.3	ABF_1 ♀♀	94	89.4
BAF_1 ♂♂	99	80.8	BAF_1 ♀♀	83	88.0
ABF_2 ♂♂	90	75.6	ABF_2 ♀♀	90	65.6
BAF_2 ♂♂	98	57.1	BAF_2 ♀♀	98	71.4
All ♂♂	378	Average 76.4	All ♀♀	365	Average 78.6

The incidence in the two sexes is thus approximately the same, and no evidence of hormonal influence exists.

Coat color.—Heston's work, referred to above, was planned to detect any signs of linkage between certain of the common genes for coat color and the tendency to form lung tumors if any such relationship existed. The genes involved were the following pairs:

C = color	*c* = albinism
A = agouti	*a* = non-agouti
B = black	*b* = brown

No evidence for linkage was found.

NON-EPITHELIAL TUMORS

Under this very broad heading are included a large number of different types of neoplasms.

In spite of a large amount of pedigree data collected by Slye (86–92) and others, there still is lacking a sufficient number of animals with any one type of tumor in any one inbred line of mice to give adequate and significant

ratios with which to test the exact genetic nature of the process of tumor formation.

There are, however, certain general factors about non-epithelial tumors that differentiate their time, place and rate of origin from that of epithelial, mammary or lung tumors.

As, in the case of mammary or lung tumors, one must begin his investigation of the genetics of the incidence of non-epithelial tumors *in inbred material* unless he wishes deliberately to handicap himself.

Even in types of tumors which histologically are relatively consistent, as the adenocarcinomas of the mammary gland or lung, there are modifying and complicating influences which affect the genetic processes. This is even more applicable to the non-epithelial tumors so that, in this chapter, emphasis will be placed upon a study of a few inbred strains and their hybrids.

In this connection a general statement contained in a recent paper by Little, Murray and Cloudman (60) may be helpful. The authors, in describing the commoner types of non-epithelial tumors, state:

"Tumors of lymph cells may occur wherever lymphatic tissue is present. They generally are primary in the spleen or in the various mesenteric or other peritoneal nodes. When they are thus situated the clinical symptoms are an abdominal swelling, with hardening, frequent turgidity, ascites or generalized edema, and occasional asymmetry. Often by careful palpation the enlargement of the spleen or the presence of other peritoneal nodules can be detected. In some animals the peritoneal cavity may be grossly distended by fluid. This fluid is of three types. It may be hemorrhagic, of the deep color of venous blood; in other cases it is milky with a pink tinge; while in still others it is clear and watery. As yet no consistent correlation between any of the three types of fluid and any particular character of the lymphatic tumor has been detected.

"The next most frequent type of non-epithelial tumor is a reticulo-endothelioma of the liver. Fluid within the peritoneal cavity is rare in association with tumors of this type. Abdominal swelling occurs, however, due to the enlargement of the liver. Naturally this swelling tends to be more anterior in position than many of the masses in the lymphocyte tumor group.

"Fibrosarcomas are apt to occur in scattered sites. Those on the jaw or leg or in the dorsal or lateral subcutaneous tissue are readily discernible as hard, fixed nodules of firm texture. Those in the uterus are usually recognizable by posterior distention of the abdomen and by the presence of an irregular palpable nodule.

"Melanomas, which are rare, are usually confined to the base of the tail and are deeply pigmented.

"Osteogenic sarcomas, which have ordinarily appeared in the long bones or jaw, are superficially very much like fibrosarcomas in those regions.

"Pathological diagnosis has been obtained for all tumors included in this report."

The inbred stock on which there has been recorded the most extensive observations is the JAX C57 black stock of the Jackson Memorial Laboratory (Tables 13, 14 and 15).

Table 13

TUMOR INCIDENCE IN THE C57 BLACK STOCK AMONG ANIMALS IN WHICH THERE ARE DATA FOR THE FULL LIFE CYCLE AND FOR NON-EPITHELIAL TUMOR INCIDENCE

Type of Animal	Total Mice	Non-tumor	Non-epithelial Tumor	Epithelial Tumor	Mean Age at Death, Non-tumor	Mean Age at Death, Tumor
Breeding ♀	570	499	64 (11.22%)	10 (1.75%)	608	706
Virgin ♀	133	109	26 (19.54%)	1 (0.75%)	814	711
Males	174	142	31 (17.81%)	5 (2.87%)	720	741

It is doubtful whether any of these groups differs significantly from the others except in the mean age at death of the virgin and of the breeding female non-tumor mice. In the case of the breeding females there is a distinct suggestion of the existence of a greater mortality risk. The virgin females have definitely a greater life span than the other groups. This may account at least in part for the high incidence of tumors since the opportunity to have such tumors which occur at an average age of 711 days was well afforded by the fact that mean age of survival of non-tumor mice was 100 days beyond that figure.

With this brief introductory statement we can next consider the relation of non-epithelial tumor incidence to various factors such as extra-chromosomal influences, age, sex, etc.

Absence of extra-chromosomal influence.—In the various experiments with non-epithelial tumors there are no cases of reciprocal crosses between

high and low tumor strains that are extensive enough in the first hybrid (F_1) generations to give significant results.

Table 14
TYPES OF NON-EPITHELIAL TUMORS IN THE C57 BLACK STOCK

Type of Tumor	Incidence
Lymphoblastoma	73 (60.3%)
Endothelioma	26 (21.5%)
Fibrosarcoma	8 (6.6%)
Lymphangioma	5 (4.1%)
Osteogenic sarcoma	3 (2.5%)
Hemangioma	3 (2.5%)
Melanoma	1 (0.8%)
Reticulum-cell sarcoma	1 (0.8%)
Undifferentiated	1 (0.8%)
Total	121

It is necessary, therefore, to compare the F_2 hybrids formed from a reciprocal cross between two inbred strains. While the two strains are not

Table 15
DISTRIBUTION BY SITE OF 121 TUMORS IN THE C57 BLACK STOCK

Location of Tumor	Incidence
Spleen and nodes	75 (62.0%)
Liver	31 (25.6%)
Uterus	4 (3.3%)
Mammary line and branches	4 (3.3%)
Subcutaneous other than mammary line	3 (2.5%)
Tail	1 (0.8%)
Jaw	1 (0.8%)
Eye	1 (0.8%)
Intestine	1 (0.8%)

classifiable with complete accuracy as "high" and "low," there does seem to be a difference between them so that we can tentatively classify the dilute brown (dba) strain as "lower" in incidence of non-epithelial tumors and the

JAX C57 black strain as "higher." The F_2 generations give the results shown in Table 16.

Table 16

Generation	Origin	No. of Animals	No. of Non-epithelial Tumors	Per Cent Incidence of Non-epithelial Tumors
F_2 (C57 black ♀ × dba ♂)	Lower ♀ × higher ♂	468	61	13.09
F_2 (dba ♀ × C57 black ♂)	Higher ♀ × lower ♂	649	90	13.61

There is very evidently no sign of extra-chromosomal influence in the F_2 generations.

Relation of incidence to age.—One fact seems clear in all the experiments thus far recorded. This is the distinctly later age at which non-epithelial tumors usually appear as compared with epithelial mammary tumors.

A tabulation of the mean age at death of mammary tumor and non-epithelial tumor mice in the same stocks and their hybrids can be made from data derived from the work of Murray, Cloudman and the writer (Table 17).

Table 17

Stock and Generation*	Mean Age in Days at Death		Excess in Age of Non-epithelial Tumor Group
	Mammary	Non-epithelial	
C57 black (B)	†	706	
dba (d)	433	†	
dBF_1	575	806	+231
BdF_1	711	808	+97
dBF_2	566	706	+140
BdF_2	623	704	+81

* Female is listed first, male second, in the crosses.

† Numbers of tumor animals too small to provide a significant value for the mean age of incidence.

It is very evident for the above data that there is involved a very different set of physiological factors in the incidence of the two types of tumors.

Relation of incidence to sex.—Scattered data derived from various workers over a period of several years indicates that non-epithelial tumors

located in tissues or organs not specific to or unequally developed in the sexes are as frequent in one sex as in the other. In this respect they resemble, as might be expected, more closely the epithelial lung tumors than those of the mammary gland.

It may well be that further and more detailed observations will reveal tendencies for certain types of non-epithelial tumors to occur more frequently in one sex than in the other. Such differences will, however, in all probability be minor and secondary and will occur as a reflection of the influence of a physiological distinction between the sexes, less important than those commonly recognized as secondary sexual characters.

Relation of incidence to coat color.—The difficulty of exact studies in this field is clear. There are, however, certain indications of relationships

Table 18

Stock	Total Non-epithelial Tumor	Lipoid Tumors	Per Cent Lipoid
Hybrids of yellow × non-yellow	44	9	20.4
Hybrids of non-yellows involving same strain	199	0	0.0

between coat color of certain types and its accompanying physiology on one hand and the incidence of non-epithelial tumors on the other.

One of the more interesting of these suggestive relationships is to be found in yellow mice which have long been known to be addicted to adiposity.

In a cross between yellow and non-yellow strains of mice there were among the hybrids nine cases of lipoma or liposarcoma. These are entirely absent in other crosses involving the same non-yellow strain. The actual figures are given in Table 18.

It seems likely, therefore, that yellow ancestry introducing physiological tendencies towards excess formation of lipoid tissue provides an increased opportunity for the origin of tumors in that tissue.

Another less clearly defined but potentially interesting relationship between coat color and non-epithelial tumor formation is to be found in the incidence of this type of tumor in "intense" pigmented mice with the gene *D* as compared with "dilute" mice homozygous for its allele *d* (Table 19).

Relation of incidence to hybridization.—The possible effect on tumor incidence of crosses between strains of mice that differ widely from one

Table 19

THE INCIDENCE OF NON-EPITHELIAL TUMORS IN INTENSE AND IN DILUTE MICE IN TWO SERIES OF CROSSES

Series	Intense		Dilute	
	Mice	Non-epithelial Tumor	Mice	Non-epithelial Tumor
I	732	105 (11.4%)	236	46 (16.3%)
II	264	13 (4.7%)	115	10 (8.0%)
Total	996	118 (10.59%)	351	56 (13.75%)

another in various physiological activities has been pointed out by Little (58).

The parent strains used were:

(*a*) *Mus bactrianus*, a small, slowly maturing, relatively infertile species.

(*b*) JAX C57 black, a strain derived from *Mus musculus*, large, rapidly maturing and fertile.

The tumor incidences in these strains and in their F_1 hybrids are compared in Table 20.

Table 20

Stock	Total Mice	Mice with Non-epithelial Tumors	Per Cent Non-epithelial Tumors
Mus bactrianus	159	0	0.0
JAX C57 black	877	116	13.2
F_1 hybrids	121	48	39.7

The increase in the hybrids is striking. There was also a definite increase in multiple tumors among the hybrids (Table 21).

LEUKEMIAS

By far the most extensive and important work in this field has been done by MacDowell and his associates. It has been admirably summarized and discussed by him (67) in a recent general paper.

He describes the origin of his material which is based upon carefully controlled inbreeding over an extensive period. As he states, in one of his

inbred strains, C58, "A surprising number of animals were found at autopsy to have enormous spleens, large livers and swollen lymph nodes. They were dying with leukemia, in most cases of the lymphatic type."

Table 21*

Stock	Mice with 1 Tumor	Mice with 2 Tumors	Mice with 3 Tumors	Mice with 4 Tumors	Per Cent of Tumor Mice with Multiple Growths
Mus bactrianus	6	0	0	0	0.0
JAX C57 black	116	10	0	0	7.9
F_1 hybrids	42	10	2	1	23.6

* Both epithelial and non-epithelial tumors are included.

In this strain, among over 700 mice observed until death, the incidence of spontaneous leukemia was 90%. Since the strain was presumably homogeneous genetically, the presence of a group of 10% which failed to develop

Table 22

	Total Mice	Incidence of Leukemia	Difference ÷ Probable Error
High tumor stock C58	606	89.6%	
F_1 from high ♀ × low ♂	139	61.9%	
F_1 from low ♀ × high ♂	106	42.5%	4.5
BC derived from F_1 ♀ × low ♂	159	46.5%	
BC derived from low ♀ × F_1 ♂	96	19.8%	7.0

leukemia showed that in these mice extrinsic influences of some sort were deciding whether or not an animal became leukemic.

When males of this strain were crossed with females of a non-leukemic strain, the incidence of leukemia in the resulting F_1 hybrids was 45%.

MacDowell defines the term leukemia as a neoplastic growth of the white blood cells, differing from cancer in the fact that these cells do not remain localized but move throughout the body. He further points out that

Table 23

Type of Neoplasm	Genetic Influence Chromosomal	Extra-chromosomal Influence	Nature of Genetic Influence	Relation to Sex	Milk Influence	Hormonal Influence	Coat Color Influence
Epithelial mammary tumors	Slight but probably present	Strong	Probably dominant	Practically confined to ♀	Present	Present	Some relation to yellow
Epithelial lung tumors	Strong	Not observed	Dominant?	None observed	None observed	None observed	None recorded
Non-epithelial tumors	Definitely indicated	Not recorded	Probably multiple factors	None essential	None observed	None observed	Lipomas may be related to yellow ancestry. Dilute coat color may be related to some types.
Leukemia	Definitely indicated	Present	Probably incomplete dominance	None recorded	None recorded	None recorded	None recorded

leukemic cells are not changed blood cells but are "a special race of cells having independent origin." He states that they arise from reticulum cells by focal proliferation. Migration obscures all trace of the point of origin. By careful observation the earliest stages of this proliferation have often been detected. They are so numerous that very clearly all of the early sites of proliferation do *not* become sources of origin of the disease. This appears to be evidence against the proliferation of normal lymphoid cells or organizations as being a precursor to the occurrence of leukemia.

Evidence of extra-chromosomal influence.—It is interesting to note that in somewhat the same manner as that reported for epithelial mammary tumors, MacDowell (69, 70) has recorded reciprocal cross differences in the incidence of spontaneous leukemia in mice derived from a cross between a "high" and a "low" line (Table 22).

There is no doubt, therefore, that some influence which is extra-chromosomal in nature is operative. Further studies of the genetic behavior of spontaneous leukemia should be important.

Comparison of the Four General Types of Neoplasms

Table 23 may help to summarize some of the main points of resemblance and difference in the four main groups of neoplasms considered.

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Chapter 7

THE GENETICS OF TUMOR TRANSPLANTATION

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GENETIC STUDIES ON TUMOR TRANSPLANTATION

Under this heading will be considered (*a*) the Mendelian nature of the genetic influences determining susceptibility and non-susceptibility to the growth of transplanted tumors, (*b*) evidence of mutations in transplanted tumors, (*c*) transplantation of leukemia.

THE MENDELIAN NATURE OF THE GENETIC INFLUENCES DETERMINING SUSCEPTIBILITY AND NON-SUSCEPTIBILITY TO TRANSPLANTED TUMORS

The early work of Leo Loeb (52, 53) showed that tumors which originated in a strain of Japanese waltzing mice would grow, upon transplantation, in approximately 100% of animals of that strain. The same tumors failed to grow in an unrelated strain of non-waltzing mice. This provided material in which there was a clear cut and uniform difference in susceptibility between two strains.

Acting upon this suggestive result, Tyzzer (85) made certain carefully controlled experiments on which he reported in 1909. His results are summarized in Table 1.

From these results Tyzzer concluded that susceptibility to the carcinoma JwA was inherited but not according to Mendel's law or any other type of inheritance then known. This conclusion seemed justifiable since what looked like Mendelian dominance in F_1 had completely disappeared in F_2.

The subsequent occurrence of a susceptible animal among mixed hybrids of F_2 and more advanced generations reopened the question, however, and suggested the need of further study (86).

Table 1

GROWTH OF AN ADENOCARCINOMA OF THE MAMMARY GLAND (JwA) OF JAPANESE WALTZING MICE, IN JAPANESE WALTZING MICE, COMMON MICE AND THEIR F_1, F_2 AND F_3 HYBRIDS

Stock	+	−
Japanese waltzing mice	142	3
Common mice	0	48
F_1 hybrids	69	1
F_2 hybrids	0	54
F_3 hybrids	0	16

In 1916 Little and Tyzzer (51) reported on a larger series of mice inoculated with tumor JwA. A total of 629 mice were used. The results in the more important generations are summarized in Table 2.

Table 2

GROWTH OF TUMOR JwA IN JAPANESE WALTZING MICE, IN COMMON MICE AND IN VARIOUS HYBRIDS BETWEEN THESE TWO

Stock	+	−	Per Cent +
Japanese waltzing mice	38	0	100.0
Common mice	0	99	0.0
F_1 hybrids	61	1	98.4
F_2 hybrids	3	180	1.6
F_1 hybrids × Japanese waltzing mice	63	0	100.0
F_1 hybrids × common	0	78	0.0

The incidence of susceptible animals in F_2 required further analysis and if possible a genetic explanation.

In 1914 Little (44) had published a brief note on a type of inheritance which might occur and which would give the appearance of being non-Mendelian, although actually depending upon Mendelizing genes. It was the continuation and development of simpler experiments already recorded

and it gave somewhat striking and startling results. It depended upon the hypothesis that certain characters of an organism depended upon *the simultaneous presence of more than one Mendelizing gene.*

Certain characters of this type were already known. Since it was this hypothesis which was applied successfully to the reaction of mice to transplanted tumors it may be discussed to advantage at this time.

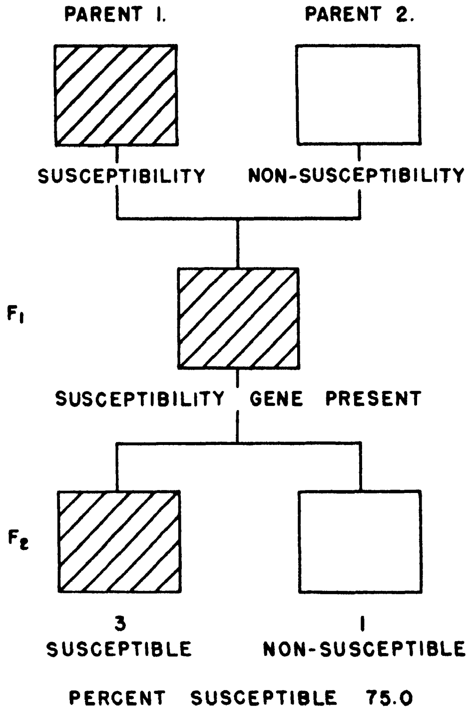


FIG. 127.—Diagram showing the inheritance of susceptibility to transplanted tumors where susceptibility is due to the presence of a single dominant gene.

Characters dependent upon one pair of genes.—It is, of course, well known that Mendelian inheritance when one pair of genes is involved gives a 3:1 ratio in F_2, in this case 3 susceptible mice to 1 non-susceptible mouse (Fig. 127).

Characters dependent upon two pairs of genes.—If now we suppose that two genes, A and B, are needed simultaneously to produce susceptibility we should have a ratio, not of 3:1, but of 9:7 or 1.3:1 (Fig. 128).

Characters dependent upon three pairs of genes.—If we continue this principle to a character dependent upon the simultaneous presence of 3 genes, the ratio will change still further (Fig. 129).

Genetic theory of transplantation.—It would be cumbersome to continue to develop this theory further by diagrams. We may, however, give a table which shows the percentages of susceptible mice to be expected when larger numbers of genes are needed (Table 3). In this table the data already shown in diagrams will be included.

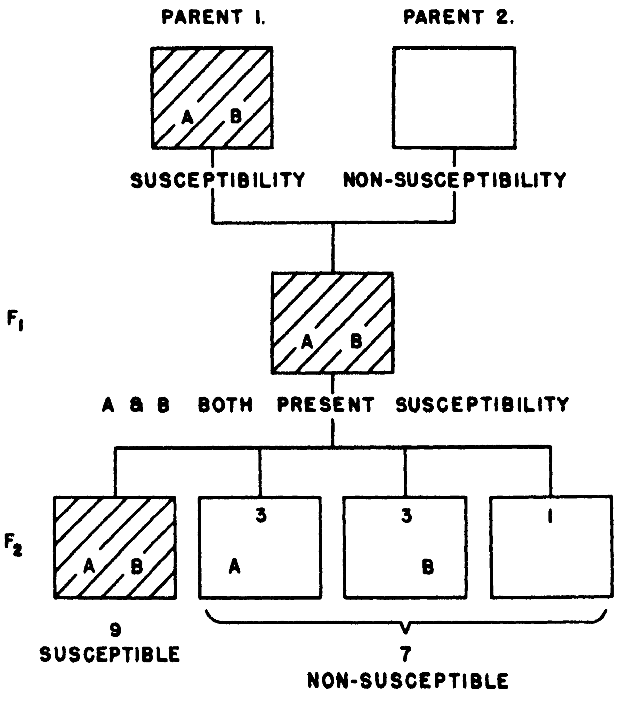


FIG. 128.—Diagram showing the inheritance of susceptibility to transplanted tumors where susceptibility is due to the simultaneous presence of two dominant genes.

It will be noted that as the number of genes needed increases the F_1 and backcross with the susceptible parent give constant figures. The behavior of the F_2 generation and of the backcross with the non-susceptible parent is quite different. As the number of genes increases the percentage of susceptible animals in these generations *decreases* with great rapidity. The decrease is more rapid in the backcross than in the F_2 generation and after 11 or 12 genes are involved would result in practically negligible occurrence of susceptible animals in the former generation.

If we now compare the results obtained by Little and Tyzzer with the expectation for 14–15 genes we get the situation shown in Table 4.

Table 3

THE RELATION BETWEEN THE PERCENTAGE OF MICE SUSCEPTIBLE TO A TRANSPLANTED TUMOR AND THE NUMBER OF GENES RESPONSIBLE FOR THE SUSCEPTIBILITY

Pairs of Genes, the Simultaneous Presence of Which Is Needed	Per Cent Susceptible in F_1	Per Cent Susceptible in F_2	Per Cent Susceptible in Backcross of F_1	
			× Susceptible Parent	× Non-susceptible Parent
1	100.0	75.0	100.0	50.0
2	100.0	56.2	100.0	25.0
3	100.0	42.2	100.0	12.5
4	100.0	31.6	100.0	6.2
5	100.0	23.7	100.0	3.1
6	100.0	17.8	100.0	1.6
7	100.0	13.3	100.0	0.8
8	100.0	10.0	100.0	0.4
9	100.0	7.5	100.0	0.2
10	100.0	5.6	100.0	0.1
11	100.0	4.2	100.0	0.05
12	100.0	3.1	100.0	0.02
13	100.0	2.3	100.0	0.01
14	100.0	1.7	100.0	0.005
15	100.0	1.0	100.0	0.002

Table 4

COMPARISON OF OBSERVED AND EXPECTED RESULTS IN GROWTH OF TUMOR JwA

	Susceptible Parent	Non-susceptible Parent	F_1	F_2	Backcross with Susceptible Parent	Backcross with Non-susceptible Parent
Observed	100.0	0.0	98.4	1.6	100.0	0.0
Expected	100.0	0.0	100.0	1.7–1.0	100.0	0.0

With this beginning as a working hypothesis, experiments were continued and extended.

A sarcoma of the Japanese waltzing mouse JwB gave simpler results indicating that from 4 to 5 genes were needed (46).

Some years later (1924) Little and Strong (50) described, in some detail, the behavior of two transplanted adenocarcinomas of the dilute brown (dba) strain of mice, dBrA and dBrB. Strong and Little (81) had previously shown that these two tumors, although apparently identical histologically,

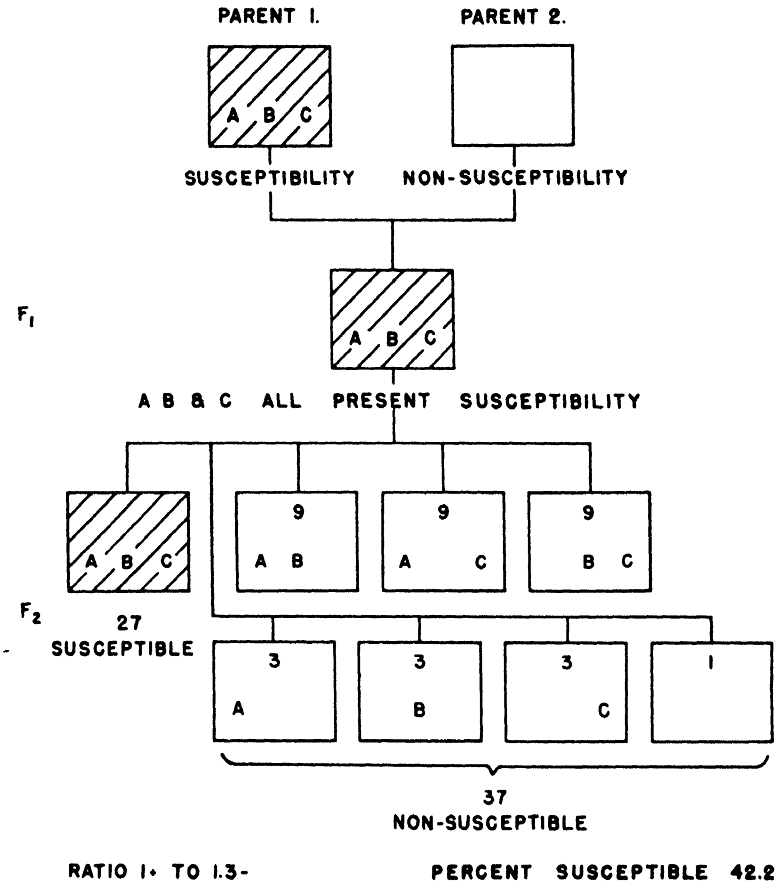


FIG. 129.—Diagram showing the inheritance of susceptibility to transplanted tumors where susceptibility is due to the simultaneous presence of three dominant genes.

gave distinctly different percentages of successful growth when inoculated simultaneously on opposite sides of the same animals.

The results of inoculating these two tumors in a large number of dilute brown mice, unrelated Bagg albino (A) mice and various hybrid generations between them are shown in Table 5.

With the exception of the backcross F_1 × Bagg albino inoculated with dBrB, the two tumors give figures which coincide closely with the expectation for two genes in the case of dBrB and three for dBrA (Table 6).

Table 5

THE GROWTH OF TUMORS dBrB AND dBrA IN TWO STRAINS OF MICE AND IN THE PROGENY OF CROSSES BETWEEN THESE STRAINS

Stock or Generation	Tumor dBrB			Tumor dBrA		
	+	−	Per Cent +	+	-	Per Cent +
Dilute brown (dba)	All	0	100.0	All	0	100.0
Bagg albino	1	130	0.26	0	131	0.0
F_1 hybrids	139	1	99.2	145	1	99.3
F_2	203	141	58.1	156	188	45.35
BC F_1 × dba	54	0	100.0	52	0	100.0
BC F_1 × Bagg	25	131	16.0	28	131	17.6

Analysis of the simultaneous reaction of the animals to the two tumors indicated that two of the three genes that were involved in the case of the tumor dBrA are the same as those which are active in the case of dBrB. The two thus bear the following relation to one another.

Table 6

EXPECTED AND OBSERVED PERCENTAGE TAKES OF TUMORS dBrB AND dBrA

	Per Cent + dba Stock	Per Cent + Bagg Albino Stock	Per Cent + F_1 Hybrids	Per Cent + F_2 Hybrids	Per Cent + Back-cross F_1 × dba	Per Cent + Back-cross F_1 × Bagg
Observed dBrB	100.0	0.26	99.2	58.1	100.0	16.0
Calculated 2 gene ratio	100.0	0.0	100.0	56.25	100.0	25.0
Observed dBrA	100.0	0.0	99.3	45.35	100.0	17.6
Calculated 3 gene ratio	100.0	0.0	100.0	42.2	100.0	12.5

Tumor dBrA needs genes A, B and C.

Tumor dBrB needs genes A, B.

These experiments helped to strengthen the probability that the working hypothesis based on Little's earlier theory was correct.

Final confirmation, however, came from two series of experiments, one by Strong (77, 78) and a still more importance piece of work by Bittner (9).

Strong's 1926 (77) paper established a "one gene" ratio in the case of a transplanted adenocarcinoma dBrCsp. This climaxed the long series of tested tumors which had begun ten years earlier.

His later paper (78) described an interesting tumor F_1Db which showed a four gene ratio in F_2. One of the genes which affected the growth of the tumor was, however, sex-linked. It thus provided important confirmatory evidence of the Mendelian nature of the susceptibility to tumor transplants.

Bittner's work was with certain transplantable tumors which occurred spontaneously in F_1 hybrid mice. It was, in some ways, related to an earlier piece of work reported by Little and Johnson (49).

In this earlier experiment splenic tissue had been used instead of tumors. Three groups of mice were used. These were (*a*) Japanese waltzers, (*b*) Bagg albinos and (*c*) F_1 hybrids between these two strains. Bits of spleen from animals in each group were inoculated subcutaneously into animals of the same group and into mice from the other two groups. In Table 7 are shown the results obtained in animals where the physical condition remained good throughout the experiment.

Table 7

THE RESULTS OF TRANSPLANTING SPLEEN TISSUE WITHIN AND BETWEEN TWO INBRED STRAINS OF MICE AND THEIR HYBRIDS

Spleen from	Spleen Inoculated into					
	Japanese Waltzers		Bagg Albinos		F_1 Hybrids	
	+	−	+	−	+	−
Japanese waltzer	81	0	0	17	33	0
Bagg albino	0	15	16	0	..	..
F_1 hybrid	0	23	.	..	33	0

The F_1 spleens failed to grow in the Japanese waltzers, thus showing that they were characteristic of hybrid animals. On the other hand they grew in other F_1 hybrids as did the Japanese waltzers' spleens.

The tumors with which Bittner worked originated in F_1 hybrids between the dilute brown (dba or "D") strain and an albino (A) strain derived from Bagg albinos (Fig. 130).

The most important and critical series of crosses were those in which tumor 13714Bx, originating in an F_1 generation mouse, was used for transplantation.

This tumor, inoculated in F_2 generation mice gave 94+ : 250−. This indicated that either 4 or 5 genes were involved. The experimental results lie between the expectation for this number of genes and afford no basis for choice as to the exact number.

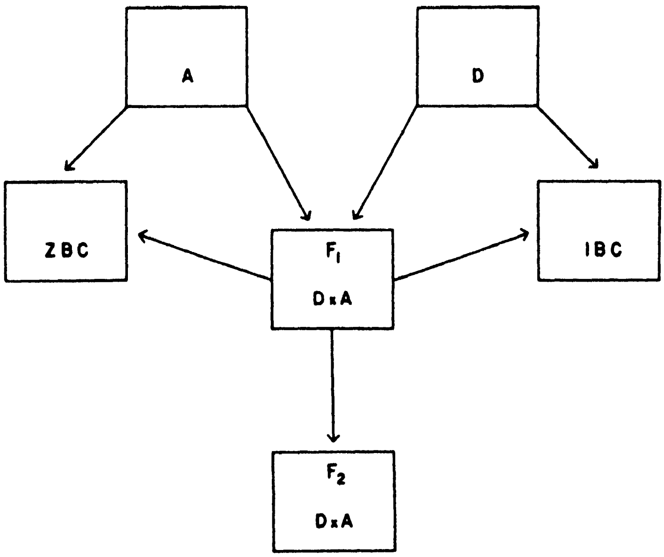


FIG. 130.—Diagram showing the relationship existing between the various stocks and generations of mice employed by Bittner in studies of transplantable tumors arising in hybrid mice. The two parent strains are called "A" (albino) and "D" (dba or dilute brown). Two arrows pointing towards a square indicate that the particular generation was produced by crossing the two stocks or generations from which the arrows come. One arrow indicates that the matings were *inter se* to produce the next generation. (*From Bittner.*)

The most interesting figures come, however, from the two backcross generations, that of $F_1 \times A$ (ZBC) and that of $F_1 \times D$ (1BC). The actual figures, compared with expectation for various numbers of genes which are introduced by the respective parent stocks, are shown in Table 8.

It appears, therefore, that probably four or five pairs of genes, of which one or two are introduced by the A stock and three or four are introduced by the D stock, are involved.

The fact that the evidence provided by the backcross generations supports the probability that four or five genes are involved, is important.

It agrees with the F_2 generation results and thus goes far to establish the correctness of the theory used to explain the observed figures.

In a review of the genetics of tumor transplantation by Haldane (33) the theory herein advanced is accepted.

Table 8

COMPARISON OF OBSERVED AND EXPECTED TAKES OF TUMOR 13714Bx, ORIGINATING IN AN F_1 MOUSE, IN ANIMALS PRODUCED BY CROSSING F_1 MICE TO THE PARENT STOCKS

Generation	Observed		Expected 1 Gene		Expected 2 Genes	
ZBC	34+	434−	29.75+	438.75−	58.5+	409.5−
			Expected 3 genes		Expected 4 genes	
1BC	23+	44−	16.75+	50.25−	33.5+	33.5−

More recent experiments by Gorer (29, 30) have provided evidence that in the case of certain transplanted mouse tumors the genes involved have a chemical basis in certain iso-agglutinogens which have been identified.

EVIDENCE OF MUTATIONS IN TRANSPLANTED TUMORS

Once the principle of Mendelizing units underlying transplantation of tumors was established, a means was provided for interpreting more accurately the nature and significance of variations in the number or percentage of successful "takes" in difference generations or experimental groups of mice.

Utilizing very cleverly selected inbred lines of mice and their hybrids, Bittner (10) was able to explain and to reproduce at will the complicated curves on which the investigators at the Imperial Cancer Research Fund in London had based a theory of fluctuating virulence of the tumor which was supposed to be rhythmic.

Bittner showed that different proportions of various genetic types was all that was required. There was no need of hypothesizing either fluctuating virulence or rhythm in that fluctuation.

It was, however, desirable to set up a series of experiments designed to show whether transplantable tumors *did* change and if so in what respects.

In order to provide the proper conditions for such a test it was necessary to keep constant the genetic constitution of the various populations studied so that when variation occurred it would be due to some change in the tumor itself.

Table 9

GROWTH OF THE TRANSPLANTED TUMOR dBrC AND OF THREE SUB-STRAIN TUMORS DERIVED FROM IT BY MUTATION

Name of Tumor		F_2 Generation	Number of Individuals	Per Cent Negative	Difference between
Original dBrC	1	Observation	23 +:102 − ± 2.89	81.60 ± 2.30	
dBrCm	5	Observation	99 +: 65 − ± 4.17	39.64 ± 2.53	1 and 5 41.96% ± 3.42 or 12.26 × P.E.
	6	Expected 1 factor	123.0+:410 − ± 3.55	25.00 ± 2.16	5 and 6 14.64% ± 3.32 or 4.41 × P.E.
	7	Expected 2 factors	92.2+: 71.7− ± 4.23	43.75 ± 2.57	5 and 7 4.11% ± 3.60 or 1.14 × P.E.
	8	Expected 3 factors	69.2+: 94.8− ± 3.60	57.81 ± 2.20	5 and 8 18.17% ± 3.35 or 5.42 × P.E.
dBrCsp	9	Observation	75.0+: 23 − ± 2.79	23.47 ± 2.86	1 and 9 58.13% ± 3.67 or 15.84 × P.E.
	10	Expected 1 factor	73.5+: 24.5− ± 2.85	25.00 ± 2.90	9 and 10 1.53% ± 4.07 or 0.37 × P.E.
	11	Expected 2 factors	55.1+: 42.9− ± 3.27	43.75 ± 3.36	9 and 11 20.28% ± 4.41 or 4.59 × P.E.
dBrCX	12	Observation	243.0+: 2.00− ± 0.94	0.82 ± 0.38	1 and 12 80.78% ± 2.48 or 32.57 × P.E.

Strong (75), working with an adenocarcinoma dBrC which originated in the dilute brown (dba) strain, found that it grew in all animals of that strain which were inoculated. It also grew in 180 F_1 mice produced by a cross between strain dba and A albinos. The F_2 generation gave a ratio (line 1, Table 9) which indicates that probably six genes were involved.

During routine inoculations of this tumor a very rapidly growing sub-strain of it was observed. This was designated as tumor dBrCX (line 12 in Table 9). From this tumor two further sub-strains seeming to show a difference in growth rate and specificity were isolated. These were called dBrCm and dBrCsp. They were carefully tested with F_2 animals and gave results shown in lines 5 and 9 of Table 9. There had evidently been a genetic change from a probable six factor tumor to two factors in the case of dBrCm and to one factor in the case of dBrCsp.

Similar results with other tumors have later been described by Bittner (7) and by Cloudman (22). In every case the change has been in the direction of decreased specificity and there have been ratios indicative of fewer factors after the change than before it.

Since the changes appear to be sudden and since they are perpetuated from one cell generation to another, they are properly definable as *mutations*. It will, of course, be necessary to discover a method of identifying the genes borne within the tumor cells before the mutations can be considered as established "gene" mutations. They are, however, abrupt genetic modifications which are self-perpetuating.

Transplantation of Leukemia

One of the most extensively studied types of neoplasm in mice is the series of leukemias reported by MacDowell and his associates.

An excellent discussion and review of this field has been given by MacDowell (57). In all of his work he has employed significant numbers of inbred genetic strains which provide authenticity and a sound foundation for future investigation.

Having demonstrated that the establishment of a true leukemic condition depends upon the multiplication of an invasion by inoculated leukemic cells, the parallel between that situation and the growth of transplanted tumors is established.

The elimination of extra-cellular agents including bacteria further strengthens the similarity of the two processes.

There also exists a high degree of specificity within the inbred genetic strain so that transplantation of leukemic cells within the strain is uniformly

successful while transfer to unrelated strains is unsuccessful. This, of course, also applies in the various transplanted tumors of mice which have been studied.

It is perhaps in the field of immunization that many of the most interesting and important contributions by MacDowell and his associates have been made.

Although they recognize that much remains to be explained, and that many complicating factors serve to obscure the true nature of the process, they have made very definite progress to which some brief reference may be made.

By introducing very small numbers of leukemic cells—as, for example, 1/4,000,000 of the standard dose—the mouse may survive. If it does, it shows that it can become modified to tolerate increasingly larger doses of cells until it is finally able to "overcome massive doses of leukemic cells given repeatedly." It is, however, clear that this immunity has no effect upon any tendency to form spontaneous leukemia in the same animals.

It is also interesting to note at this point that often the first transplants of leukemic cells derived from a spontaneous growth, will not kill the host before 20 to 90 days, while after a long series of transfers from mouse to mouse, death may result in 3 or 4 days. This has also been the history of *some* transplanted tumors, but not of all.

In such "immunized" mice there is no trace of any antibodies in the serum. This is in accord with the results obtained with transplanted tumors.

Although there are no antibodies present "a susceptible host can be immediately protected against a lethal dose of leukemic cells by treatment with minced tissue from an actively immunized animal." Whatever causes this protection is "intimately associated with living cells." By forcing the minced tissue out of a syringe "held firmly against the bottom of the vessel" all the cells are torn apart and the protective property is destroyed. Very evidently these facts raise the possibility that some unknown mechanism of resistance is involved.

The injection of entirely normal tissue from an unrelated mouse may also confer immunity. Various organs, both embryonic and adult, may be used. Genetic constitution has a role to play. MacDowell states, however, that "before making the test there is no means of knowing the effect of normal tissue of a given genetic constitution, except that the tissue of the same genetic constitution as the host is ineffective."

The resistance produced by a single treatment with normal tissue "differs from that induced by leukemic cells in that it cannot be passively transferred

to another host, and, while regularly delaying the progress of leukemic invasion, does not always give lasting resistance." There may be delayed appearance of leukemia or "curious subcutaneous tumors may appear with the histological characteristics of lymphosarcoma." Such tumors have not been obtained elsewhere. When transplanted into normal hosts these peculiar tumors give rise to leukemia of the same type peculiar to the line of leukemic cells previously inoculated. In some cases, however, the inoculated lymphosarcoma type of tumor reappears in one or more transplant generations in untreated hosts. This suggests a different type of resistance mechanism on the part of normal tissue to that of leukemic cells.

The importance of continued studies in this field is thus obvious and should be generally recognized.

With the general conclusion that the genetics of tissue transplantation has a Mendelian basis, and that the number of genes involved varies in individual cases according to the degree of genetic similarity or difference between donor and host, we may consider certain of the more practical aspects of tumor transplantation.

Practical Considerations

Methods of Transplantation

The commonest method of transplantation is by use of a trocar. In this and all other types, great care to maintain asceptic conditions should be taken. An infected tumor or site of implantation results in the introduction of factors which importantly influence the continued growth of the implant. A tumor when removed under asceptic conditions and placed in a sterile dish may be cut into a number of small bits. These may be loaded in a trocar one at a time and by a blunt plunger be pushed out through the sharpened end of the trocar after that has been inserted to the site at which the implant is desired. This method can be used for subcutaneous or intraperitoneal implantation. In the case of the former, the trocar can be withdrawn through a constriction formed by grasping the skin with the forefinger and thumb just above the tip of the trocar, thus preventing the implant from being pulled out of place.

Another method closely allied to the above is the implantation of bits of tissue by fine pointed forceps. This method may at times possess certain advantages of greater accuracy in location of the implant. With the exception of the instrument used, it varies little, however, from the trocar method.

The use of a fairly coarse hypodermic needle is often helpful. In this case the sterile tumor, after removal, is cut into bits which are then ground

into a mush by mortar and pestle. If an emulsion which will pass through a fine needle is desired the process of grinding must, of course, be more prolonged and careful. Either normal salt solution or Ringer's solution may be used as a medium for thinning the emulsion. This method is naturally more delicate than either of the foregoing and is valuable in reaching relatively inaccessible or restricted sites.

Sites of Transplantation

The ear.—This provides a site easy of observation. There is a relatively slight blood supply, however, and the temperature is apt to be below that of the peritoneal cavity or various subcutaneous sites. For this reason the ear is a favorable site for testicular transplants.

The forehead.—This is a convenient site and one in which the opportunity for invasion of underlying tissue is definitely limited by the proximity of the skull. The blood supply is relatively low.

Subcutaneous axillary and inguinal.—The paired sites thus provided are very frequently used. The blood supply of both areas is good, that of the axillary region being the better. In using these sites it is well to make the incision through which the trocar or the forceps are inserted at some distance at some lateral location.

Subcutaneous mid-dorsal and mid-ventral.—These are also frequently used. Accurate location of the implant is more difficult than in either the axillary or inguinal sites, but the blood supply is good.

Subcutaneous tail.—This region provides the lowest blood supply and slowest growth of any yet studied. It is advantageous because of ease of observation and because the tail can be wholly or partially removed, thus providing a convenient aid in studies of induced immunity.

Intracranial.—This site can be approached after removal of a small amount of bone which can later be replaced or through a fine hole drilled in the skull. It can also be utilized by the careful insertion of a small hypodermic needle if solutions are used for the implant. Its advantages are those common to the site in other forms. There appears to be in this site an unusual degree of non-specificity. There are several records of successful transplants of mouse tumors in rats, guinea pigs or even pigeons. Quite obviously extensive growth of any implant is accompanied by serious symptoms and disturbances.

Intraperitoneal.—Mice are particularly resistant to infection and to operative shock so that any site in the peritoneal cavity is available with comparatively little danger or difficulty. Suspension of tumor cells injected

into the peritoneal cavity often gives rise to many small nodules of healthy tissue convenient for reinoculation.

Intrathoracic.—This cavity can be reached either through the diaphragm or the intercostal spaces. The postoperative results are usually satisfactory.

Intra-uterine.—Because of the small size of the os and the danger of tearing it if inoculation through it is attempted, it has been found that exposure of the uterus by abdominal operation, by either dorsal or ventral incision, is preferable. The uterus provides an excellent site for transplantation.

Intratesticular.—By maintaining pressure which keeps the testis in the scrotum it becomes easily available as a site for implantation. If a more delicate technique of transplantation is desired, an abdominal operation is simple and effective.

Intravenous.—The most accessible site is the tail vein in which inoculations can easily be made. By proper care and experience this vein can be used many times in a series of experiments without great difficulty.

The eye.—In rabbits a satisfactory technique for implanting bits of tumor tissue in the anterior chamber of the eye has been developed. In this site vascularization appears to be rapid and extensive. Possibly as a result of this factor alone or in combination with decreased specificity of reaction to foreign tissue in that area, successful growth of homologous and even of heterologous tissue has been reported. As yet this technique has been little used in mice although it offers real promise.

By application.—If desired a bit of tumor tissue may be applied to the surface of an organ or tissue and be held in place there either by some adhesive membrane such as that formed by collodion or by a single suture. This method has the advantage of affording an opportunity to study surface reactions between any two tissues.

MATERIAL USED IN TRANSPLANTATION

Since new tumors are constantly being discovered and are being used for transplantation, it is impossible to make, at any one time, a complete and permanent list of this material.

On the other hand, it may be helpful in giving a picture of the opportunities for research in this field if some of the more interesting and commonly used tumors are mentioned.

IMPERIAL CANCER RESEARCH FUND, LONDON, ENGLAND

No. or Symbol	*Type of Tumor*
27	Adenocarcinoma of the mammary gland
37S	Spindle cell sarcoma

No. or Symbol	*Type of Tumor*
63	Alveolar* carcinoma of the mammary gland
91	Alveolar and adenocarcinoma of the mammary gland
113	Alveolar carcinoma of the mammary gland
155	Adenocarcinoma of the mammary gland
173	Tar carcinoma—undifferentiated squamous
206	Alveolar carcinoma of the mammary gland
2146	Tar carcinoma—polymorph
Twort	Alveolar carcinoma of the mammary gland
Melanotic Harding-Passey	Melanotic sarcoma—unpigmented strain
3187	Mast-cell sarcoma
Berlogh	Anaplastic carcinoma (Originally from Silberstein Vienna as Ehrlich mouse carcinoma)
B.P.	Sarcoma—benzpyrene in subcutaneous tissue
Oestrin	Mammary carcinoma

COLUMBIA UNIVERSITY, NEW YORK, N.Y.

Ehrlich Chondroma	Received direct from Frankfort 1924—slow growing
Sarcoma 37	Originally 37S from London. Polymorphous cell
M180 (Crocker 180)	(1914) Polymorphous cell sarcoma
M2163	(1938) Left axilla—undifferentiated carcinoma with some areas of adenocarcinoma

HUNTINGTON HOSPITAL, HARVARD UNIVERSITY, BOSTON, MASS.

Ovarian embryoma	(1938) Ovarian embryoma originating in C_3H mice

YALE UNIVERSITY SCHOOL OF MEDICINE, NEW HAVEN, CONN.

Brain tumor	Meningeal sarcoma (obtained from Drs. Seligman and Shear)
Brain tumor	Glioma (obtained from Drs. Seligman and Shear)
Rhabdomyosarcoma	Obtained following injection of methylcholanthrene, C_3H stock
Hepatoma	Originated in CBA stock
Ovary	Carcinoma, CBA mice

Many types of tumors induced by hormones or synthetic chemical carcinogens are usually available.

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* The term "alveolar" is used in this connection to denote tumors having solid masses of cells in contrast to the glandular or adenocarcinomatous type.

Dr. Margaret Reed Lewis and Dr. Warren Lewis, Wistar Institute, Philadelphia, Pa.

No. or Symbol	*Type of Tumor*
6 sarcomata	Transplantable in Bagg albino mice
3 sarcomata	Transplantable in Little C_3H stock
5 sarcomata	Transplantable in Little C57 black stock
1 sarcoma	Transplantable in Murray dba stock
4 white blood cell tumors	Myeloid cells, 3 in dba and 1 in C57 black
4 white blood cell tumors	Lymphoid cells, 3 in dba and 1 in C_3H
2 white blood cell tumors	Monocytic, 1 in A stock and 1 in C57 black

Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Me.

15091a	Spindle cell carcinoma of the mammary gland (1928) originally diagnosed by Ewing and Warthin as adenocarcinoma. Thirteenth generation became carcinoma simplex. Twentieth generation gave evidence of transformation of epithelial cells to spindle cells. A stock
L946A II.	Fibrosarcoma originating in osteogenic sarcoma of the tail (1936). C57 black stock. No bony elements
E060	Papillary adenocarcinoma of the mammary gland (1936) C57 black
C617	Adenocarcinoma of the mammary gland (1938) C57 brown stock
dbrB	Adenocarcinoma of the mammary gland (1920) dba stock
S91	Melanoma (1937) primary at base of tail dba stock
C252	Fibrosarcoma (1936) subcutaneous pelvic region C57 leaden stock
C198	Reticulo-endothelioma liver—rare type (1936) C57 leaden stock
P208	Melanoma (1937) on side of dba strain mouse
P764	Embryonal cell carcinoma of the testis (1939) dba strain

From the above list the great diversity of available material will be evident.

Practical Suggestions

The following suggestions are made to those who desire to utilize genetic knowledge in the transplantation of tumor tissue in mice.

1. For routine carrying on of tumors use one or more strains produced and maintained by brother to sister or parent to offspring matings. Use either the strain in which the tumor originated, which should give approximately 100% takes, or if this is impossible, any inbred strain that gives a high proportion of positive animals.

2. For routine carrying on of tumors at rapid rate of growth, maintain one or more pure strains as above indicated. Use animals from such strains to cross with one or more unrelated inbred strains to produce first generation (F_1) hybrids. Use these for inoculation. They usually grow the tumor more rapidly than the inbred animals themselves.

3. To use an inoculated tumor as a means of measuring the degree of physiological difference or similarity between strains several steps are necessary: Maintain two or more distinct inbred strains, one of which is the strain in which the tumor originated, the other being the strain or strains which are to be compared with it.

4. The storage of tumor tissue in dry ice refrigerators (about −70°C.) has also proven a satisfactory method of preserving this type of tissue. Best results have been obtained when the tissue is frozen slowly, thawed rapidly. Some investigators have found this method satisfactory for all types of tumors, others have reported success with some tumors but unreliable results with others.

Transplantation of Genetically Controlled Tumors in Relation to the Study of Growth and Individuality

Factors Influencing Successful Transplantation

In addition to the genetic constitution as an important factor in determining success or failure of transplanted tissue there are a number of other things which may influence the final result.

Among these, several may be briefly discussed.

Diet.—Various experimental, unbalanced and defective diets have been reported as influencing the number of "takes" and the rate of growth of transplanted tumors. There is no doubt that diet may play a part in determining the reaction of the animal. On the other hand, the fact that the investigators have not used inbred strains to reduce and control the genetic variables, leaves it uncertain as to the cause and effect relationship between diet and the changes in percentage of growth. This fact, coupled with an almost complete disregard of criteria of mathematical significance between

the groups that are being compared, seems to have left the problem of diet in a most unsatisfactory condition. For this reason no attempt is made in this volume to cover the extensive but non-critical bibliography. The whole problem will have to be approached "from the ground up" by investigators who understand and utilize genetics, biochemistry and mathematics.

Irritating agents.—There have been several types of experiments dealing with the effects of irritants of various sorts in the response of an animal to implants of tumor tissue.

Perhaps the simplest approach to this problem is through the introduction of a mechanical irritant which is not able to exert any evident chemical reaction.

A series of experiments of this sort was reported by E. E. Jones (37) who found that growth of an adenocarcinoma was obtained in a number of mice belonging to stocks, otherwise negative, when a bit of sterile non-dyed flannel was inoculated with a bit of the tumor.

This interesting result indicated that possibly local factors as well as those affecting general lymphocytic reaction may be operative.

It would seem that further study of this general field would prove fruitful.

It is also known that previous exposure of transplantation sites to physical agents such as heat, cold or radiation may affect the percentage of successful implants and their rate of growth. As yet, however, data on these effects are so fragmentary and diffuse as to prevent any general conclusions being drawn. Biochemical irritants of some types have also been used.

Perhaps a typical and interesting result is that obtained by Koenigsfeld who found that animals painted with carcinogenic tar and inoculated at the same time with a transplantable tumor showed increased response to the former and more rapid growth of the latter. This interesting mutual activation remains unexplained and is in contrast with the experience of investigators who have compared the interaction of centers of benign growth with a center of malignant growth. In this case the usual experience has been that pregnancy slows the rate of growth of transplanted cancer except in the case of certain exceptional tumors. These are mammary adenomas which in some instances have grown more rapidly when the host is pregnant than at other times.

In all of these experiments, as in those dealing with dietary factors, the present need is for a more accurate control of the too numerous variables which, influencing the fate of the transplant, may serve to mask or to distort the relationship between any one experimental factor and the end result.

Age, sex and other biological factors.—In 1920 Little (45) showed that temporary growth of tumors destined to eventual regression and disappearance was more readily obtained in very young animals than in young adults. Strong later showed that the same is true of very old animals as compared with those in the prime of physiological activity.

A difference in the rate at which the sexes acquired the ability to eliminate transplants of tumors was also demonstrated by Little (Table 10).

Table 10
DIFFERENCE IN THE RATE AT WHICH THE SEXES ACQUIRE THE ABILITY TO ELIMINATE TRANSPLANTED TUMORS

	Age in Days at Inoculation	Observations Showing Mass	Observations Negative	Per Cent Showing Mass	Diff. ± P.E.	Diff./P.E.
Males	2–10	36	212	14.51 ± 1.51	0.87 ± 2.16	0.4
	12–20	38	209	15.38 ± 1.55		
Females	2–10	33	231	12.12 ± 1.76	13.16 ± 2.71	5.0
	12–20	52	150	25.74 ± 2.07		

In this case the mice used were those of a hybrid generation in which some of the animals would presumably show progressive *growth* of the implants and others (the majority) would show regression and eventual disappearance. The female mice in the older age group gave a significantly higher percentage of "takes" than did the males. This was in all probability due to the earlier assumption by some of these animals of the biological make-up which reflects the presence of genetic factors for susceptibility. Female mice mature distinctly more rapidly than do males. They would, therefore, begin earlier to express their characteristic genetic constitution. This actually is the case.

As a contrast to the hybrid mice among which are to be found a number of animals with a genetic constitution favoring susceptibility, may be cited the results of inoculating females of a completely non-susceptible strain. These results are shown in Table 11.

Here it will be noted that the non-susceptible genetic constitution is expressing itself rapidly and definitely in a significant *decrease* in positive observations.

Complete or partial castration and ovariectomy have also been studied in relation to growth of transplanted tumors. The results obtained by different investigators have varied as have the conclusions drawn from them. This is probably due to the fact that various stocks, ages and tumors have been used. An additional variable has been provided in the interval between operation and implantation of the tumor.

One of the most complete and careful studies of this question has been made by Strong (72). He concludes:

1. Removal of the gonads does not change the massed percentage reactions for individuals of a non-susceptible race. This bears out the previous conclusion that the number of percentage reactions in a given strain depends upon the genetic constitution of the individuals.

Table 11

Age in Days of Females at Inoculation	Observations Showing Masses	Observations Negative	Per Cent Showing Masses	Diff. ± P.E.	Diff./P.E.
2–10	80	331	19.46 ± 1.31	10.7 ± 1.51	7.0
12–20	48	463	9.39 ± 0.87		

2. Gonadectomy produced, in the stock employed, a significant increase in percentage reactions in mice attaining sexual maturity.

3. Gonadectomy causes an approach towards a "neutral" type (loss of characteristic differences between sexes) in the percentage of reactions—just as it does in the case of morphological characteristics.

4. By the removal of the gonads, the individuality of tissues and the normal functioning of the age factor can be interfered with.

5. A severe shock caused by such an operation as gonadectomy produces, in some cases at least, a resistant state to transplantable tumors, that is at its maximum from five to ten days after the operation.

Other investigators have found similar shock effects following operative removal of the spleen.

In all of these physiological studies a common criticism can be made. It is roughly the same as that applied to investigations of diet; namely, that too little work has been reported on material in which the number of variables has been reduced to a minimum.

It will be necessary to wait until far more extensive and carefully controlled studies have been made before any conclusions of general application can be drawn.

Relation to Individuality

Transplantation studies afford one of the most promising methods of investigation of the process of acquisition of complete biological individuality.

By the growth of heterologous adult tissue in embryonic culture media, such as the allantois of the chick embryo, and by the opposite process of growing embryonic tissue for a considerable period in heterologous adult individuals, evidence is clearly provided that full expression of the biochemical characteristics of the species, strain or individual is gradually developed.

Tumors which represent a source of supply of rapidly growing tissue in which the degree of biochemical specificity may, to some extent, be measured by genetic tests are valuable aids in such research.

By holding the source of tumor material constant and by varying the degree of biological differentiation of the host that receives the implant, information concerning the process of differentiation both chemical and morphological should be obtained.

Similarly by the inoculation of several types of tumors in a single host the reaction of that host can be measured in terms of its response to different biological stimuli.

The Relation of Transplantable Tumors to Spontaneous Tumors

The bearing of genetic work with transplanted tumors on the genetics of spontaneous tumors in mice is one on which a great deal of difference of opinion exists. One of the commonest points of view is that a clear and distinct line should be drawn between experimental work on (*1*) transplanted tumors, (*2*) induced tumors and (*3*) spontaneous tumors. While there is no doubt that characteristic differences exist between the three groups as regards the type of problem which each is best fitted to cover, it seems likely that an extreme point of view such as that cited is incorrect. One of the reasons why a point of view of that sort has developed is that there is proper objection to applying, *in toto*, the results obtained with either transplanted or induced tumors to the field of the spontaneous tumors. This does not mean, however, that work with spontaneous and induced tumors may not

contribute definitely to our understanding of the processes of formation and growth of spontaneous neoplasms.

One principle may safely guide us in this discussion. It is the fact that only those who have had direct and continuing, first-hand knowledge of experimentation in all of the three fields are qualified to evaluate with any degree of probable accuracy the relationship between them. This again does not mean that the student of transplanted tumors alone may not contribute greatly to our knowledge of the cancer process. The same, of course, applies to investigators who use only induced tumors or who study only spontaneous tumors. All that is meant is that relations between the three types of experimentation are best understood by those who have engaged in all of them.

With this preliminary discussion we may consider briefly three principles established by abundant experiments with transplanted tumors which have an important bearing on the problem of spontaneous tumors. These have been considered in a paper by Little (48). They are as follows:

1. Transplantation in known and controlled genetic material provides a more delicate test of biological and physiological differences between certain neoplasms than does any other test at present available.

2. Transplantation experiments in which somatic mutational changes in the genetic constitution of a tumor have been demonstrated afford a most helpful avenue of investigation on the nature and incidence of somatic mutation as a process of importance in cancer research.

3. Transplantation experiments on the genetics of spontaneous tumors arising in F_1 and other hybrid mice, derived from a cross between two inbred strains, give an unusually good opportunity for linkage studies between tumor genes, derived from the parent races, and genes for other characters of a more easily detectable nature. They also should enable us to determine whether hybridization as a process has any influence on the genetic complexity of tumors formed.

In each of these cases transplantation is being used as an experimental method as an aid in analysis and not as a process which creates important facts *de novo*.

Transplantation and the physiological individuality of tumors.—In 1920 Strong and the writer (81) published evidence which showed that two mammary adenocarcinomas of the mouse, although histologically indistinguishable, gave very different percentages of continuing growth when inoculated into hybrid mice of known genetic origin. The rate at which these two tumors were eliminated by a negative strain of mice also showed a

clear and persistent difference. The amount of temporary growth which each exhibited was also different. The use of stocks of mice in which temporary growth of transplanted neoplasms is followed by regression gives a very delicate physiological test of the nature and activity of that tumor. A series of tumors compared in this way often reveals more subtle and minute differences than are detectable by any other known test. Cloudman has made an intensive comparative study of the transplantation of mammary tumors arising spontaneously in a single mouse and has shown that in the case of three adenocarcinomas of the breast very different genetic constitutions were involved. These tumors appeared at essentially the same time. It is clear that the transplantation test provides a method of determining whether these three tumors were independent primary growths or metastases of the same primary neoplasm.

It is also evident that by a comparison of the genetic factors in such a series of tumors much information can be derived as to the factors which all possess in common and those which are specific to a single growth. It is quite conceivable that if extensive studies of this type were made we might, by plotting the relationships of the genetic factors, obtain a valuable picture of the process of tumor formation as a whole from a biological point of view.

Similarly during the lifetime of an individual successive neoplasms occurring at intervals as the age of the animal increases may be maintained through transplantation and studied in comparison with one another to find out whether older animals give rise to tumors which are characteristically different from those produced by younger ones. All this type of work in its various implications should contribute very definitely to our knowledge of the process of disintegrating individuality in ageing animals.

Transplantation experiments and somatic mutation.—The question of somatic mutation is discussed further in Chapter 6. For the present it will suffice to point out that the occurrence of mutations in transplanted tumors which increase the percentage of takes of these tumors is a well-established phenomenon supported by the work of Strong, Bittner and Cloudman. Ordinarily in tumors involving a number of genes these changes also affect more than one gene, also there have been cases where apparently a change in a single gene resulting in a change from a two factor to a one factor ratio has been observed. In these tumors the mutational change is clearly somatic since the tumor in which the changes occur is composed of somatic and not germinal tissue. Tyzzer and many others subsequently have suggested that the change from a normal to a tumor cell may be in

the nature of a somatic mutation. The question is still undecided, but it is certain that some of the most favorable material in which to study it is to be found in the modification of transplantable neoplasms. Such tumor tissue can be subjected in various amounts to chemical and physical conditions which have been shown to be mutation producing agents. Changes in the tumor can afterwards be studied and recorded. Controlled series of normal tissue subjected to the same agents can be maintained.

Treatment of various clearly defined sites in animals of known tendency to produce spontaneous tumors of different types with agents likely to produce mutation should give interesting information as to whether these agents increase the incidence of spontaneous cancer. If they do so the relation of this increase to the higher mutation rate in germ cells affected by similar agents should be interesting and important.

Genetic studies of tumors originating in hybrids.—Transplantation studies of tumors of this type should add more knowledge to the genetic analysis of spontaneous tumors by providing evidence for linkage between genes which underly the growth of transplanted tumors and some other known Mendelizing genes. As the number of known genes in mice increases and linkage becomes more generally recorded, the chance of finding genes related to the process of spontaneous tumor formation should similarly increase. If there is no evidence of such linkage when it may fairly be expected, the negative findings will themselves be important in determining the relative roll of chromosomal inheritance and other etiological factors in spontaneous tumor formation. Preliminary evidence of linkage between genes determining the growth of certain spontaneous tumors and those for certain types of coat color has already been obtained. The need of obtaining rapidly the largest possible number of genes is evident and the field of tissue transplantation (more particularly that of tumors) should give us valuable new information.

At all events, the genetic analysis of transplanted and induced tumors has a direct and permanent bearing on similar studies with spontaneous neoplasms.

Relation to Transplantation of Normal Tissue

Little has been said in this chapter on the bearing of tumor transplantation to the genetics of normal tissue transplants.

This omission is not due to the fact that the subject lacks importance or interest. It results from the somewhat extraordinary fact that so little

work has been done in this field—with properly controlled material—that it remains practically an open door for experimentation.

Up to the establishment of the Mendelian nature of the genetic factors influencing growth of transplants, Loeb had presented the only comprehensive theory to attempt to explain success or failure of implants of normal tissues. In 1924 Little (47) reviewed and criticized Loeb's work up to that point. Discrepancies between experimental results and Loeb's theory were pointed out.

Later Loeb and Wright (56) and Loeb and King (55) investigated the transplantation of normal tissues in inbred and hybrid strains of guinea pigs and rats. The data obtained from these experiments were in agreement with the genetic theory of transplantation of tumors as given earlier in this chapter.

So also were the results of Bittner (17) working with mice.

We may, therefore, conclude that distinct advances in our knowledge can be made when further studies along these lines have been conducted.

Conclusion

In conclusion we may point out the fact that few investigators as yet recognize and utilize the great opportunities for new attacks on many basic biological problems afforded by the recent advances in our knowledge of the genetics of tissue transplantation.

With inbred strains of mice now available there is a whole new field of attack, not only on the problems of experimental cancer, but on those of the nature of individuality and of the fundamental processes of ontogeny.

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Chapter 8

ENDOCRINE SECRETION AND TUMOR FORMATION

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The secretions of those glands which liberate their substances into the blood and lymph have a marked and important relation to tumor formation in mice. This has been well demonstrated but only a few of the probably many details of these relationships are known at the present time. For mammary gland tumor production the presence of a certain type of hormone is an indispensable factor. In addition to furthering the problems arising from the primary differences due to sex itself, there are many possibilities for extension of knowledge in this field. Strains of mice which develop different but comparatively uniform percentages of mammary, as well as other, tumors furnish excellent material for following up the indications of at least quantitative differences in the hormonal control mechanisms and of their relation to tumor incidence. The endocrine differences which limit generalization not only between species but within a species such as *Mus musculus* are no doubt important stepping stones along the path to a more complete analysis of the interrelationships. The isolation and chemical determination of many of the sex hormones, together with the synthesis of related compounds, is rapidly leading to more extensive and specific modification of hormones within experimental animals. All of these are greatly aiding the progress of our knowledge. To make clear the relation of these secretions and of related substances to tumor formation is the first step. To be able to use this knowledge to aid in the control of tumor formation is the eventual goal.

Because of the incompleteness of the picture of the relation of internal secretions to tumor formation at the present time an attempt is made only to call attention to some of the studies now available rather than to try to fit the picture together. The bibliography is not exhaustive but through its

use reference may be had to many of the original studies. The material is restricted to the mouse. In developing the review, use has been made of the divisions which the mechanics of experimentation have projected into the field. For example, those studies where no hormones have been added from outside of the body have been separated from those where an addition in one form or another has been made. Transplanted tumor studies and evidences of hormone production in tumors are in still separate sections.

With no outside addition of hormones.—Of all the types of tumors that have been found in experimental mice none have been more extensively studied or more closely related to the endocrine system than those of the mammary glands. That an endocrine factor was involved in the development was indicated by the experiments of Loeb (55, 56), Lathrop and Loeb (52) and subsequently confirmed by many experimental workers. Three facts stood out. First, mammary tumors developed spontaneously in female but never or very rarely in male mice. Second, the incidence of mammary tumors in female mice varied according to the breeding activity: virgin females had a lower percentage than parous or multiparous females. Third, the tumors of virgin females appeared later than those of breeding females.

That an endocrine factor was ovarian was indicated by the removal of ovaries at various ages, a technique which caused a decrease in mammary tumor percentage in direct proportion to the time of the ovary removal (55, 52). Experiments by Cori (15) showed that ovariectomy at 16 days of age reduced the percentage to almost zero. Further experiments with ovariectomized mice supported and added to the work of Loeb and Cori (70, 71).

Many experiments have demonstrated that the ovarian factor is not the sole factor leading to mammary tumor development. The genetic and milk influences are discussed in other chapters of this book. That the endocrine factor need not always be ovarian has recently been demonstrated (87). Following ovariectomy at birth, Jackson Laboratory dilute brown mice developed nodular hyperplasia of the adrenal cortex. This was followed by stimulation of the vagina, uterus and mammary glands. Twenty-seven per cent of the ovariectomized females developed mammary gland tumors. These changes leading all the way to tumor production are not limited just to the dilute brown strain of mice, though they do not occur to the same extent in some of the low tumor strains of mice (86). Recently mammary tumors have appeared in male mice which were castrated at birth. This again followed development of nodular hyperplasia of the adrenal cortex

and subsequent growth of the mammary rudiments into extensive duct systems.

It has been found that lymphosarcoma in one line of mice was nearly twice as frequent in females as in males (61). In another study, daughters from reciprocal matings showed the same difference in incidence of leukemias as the sons (59).

A brown degeneration occurring in the adrenal glands of both sexes of mice has been described. Efforts to correlate this degeneration with variation of estrogenic hormones and the incidence of mammary cancer have been made (16, 19).

The reproductive physiology of strains of mice with various percentages of mammary tumors has been investigated (57, 43, 44, 37, 10, 68, 7, 80). Special characteristics of the estrous cycle such as unusual duration of phases of the cycle have not been consistently correlated with tumor incidence.

It has been observed that the frequency of breeding had in some cases marked influence on the incidence of mammary tumors in mice (3, 4, 53, 25). Whether the result was related to the rapidity of the pregnancies in themselves or to the irritation of stagnating products in the mammary ducts is not certain.

In an extensive study of mice painted with tar it has been found that the males showed a distinctly delayed tar tumor reaction as compared with the females (40).

With unusual addition of hormones.—Experimental studies have shown that sex hormones can awaken malignant changes at least on a substratum that is usually thought of as hereditarily susceptible to cancer. Thus Murray (71) found that mammary tumors appeared in fifteen male mice out of 210 castrated at 3 to 4 months of age when ovaries from sisters were implanted. An inbred Jackson Laboratory tumor strain of mice was used. Feminization of the male mouse, in which the mammary rudiments undergo little if any development throughout life (30), induced growth of mammary glands and also the development of mammary tumors. This was confirmed by deJongh (21).

Following the injection of estrogen,* males from high tumor strains of mice developed mammary tumors as frequently as multiparous females (41, 42, 27, 32). Males from low tumor strains developed tumors with

* Estrogen: a generic name for female sex hormone. The term as here used is intended to include synthetic as well as naturally occurring hormones.

greater frequency than multiparous females of the same strains. Mammary gland tumors have not yet been obtained following estrogen injections in male mice from some of the very low tumor strains even though prolonged efforts have been made to produce them (8, 44, 27). It might be assumed that estrogens act in conjunction with some intrinsic factor predisposing to tumor formation. Mammary tumors were produced in males of one low tumor strain following injection of an estrogen only when nursed on high tumor mothers (84). The method of injection is of importance. Using a high tumor strain it was found that 3000 rat units of estrogen over a three day period at two weeks of age was not effective in producing mammary tumors in males but 16 weekly doses of 100 rat units each produced a high incidence of tumors (11). Synthetic estrogens which differ markedly in molecular structure from naturally occurring forms will produce tumors in male mice (48, 75). This makes it difficult to assume that there is carcinogenic action associated with hormone molecular structure (50).

Mammary gland carcinomas have developed in females of very low tumor strains following estrogenic treatment, though only after long periods of treatment (9). The incidence of mammary tumors in mixed stocks has been increased as compared to the controls (74). The incidence of mammary tumors was increased in female mice of both high and low tumor strains following estrogen injections (81).

The activity of the corpora lutea may be a contributing factor to mammary gland cancer production (58). However, progesterone alone or in combination with estrogen did not alter mammary tumor percentages (50, 28).

A considerable increase in tumor rate in non-breeding mice of several strains was observed following subcutaneous transplants of three or four anterior lobes of the hypophysis from male and female litter mates (58).

Modification of the incidence of mammary tumors in mice has been attempted with male hormone preparations. It has been reported that testosterone administered to female mice of a highly susceptible strain will result in a marked fall in the incidence of mammary tumors (51, 72). The mechanism of the inhibition is not understood although evidence has been marshalled indicating that the action is through the pituitary (50). The lowering of incidence when treatment is started with mature animals has not been suitably confirmed (50).

The appearance of mammary cancer has been prevented by use of the thyrotropic hormone of the pituitary gland (20). In another study the

same hormone failed to prevent the occurrence of mammary tumors in females, or in males treated with estrogen (49).

Cancerous lesions in or near the cervix have been reported following the injection of estrogens (45, 56, 82, 29). One of these tumors was grafted into young male and female mice in which it continued to grow without further hormone administration (29). Lesions of the cervix similar to malignant tumors appeared in mice receiving estrogen and 1:2:5:6 dibenzanthracene (74, 73).

Hemorrhagic chromophobe adenomas of the pituitaries developed in mice following long continued injections of estrogen or its cutaneous application (17, 18, 12). Hypophyseal tumors did not appear in six inbred strains following the injection of several estrogens for prolonged periods. In another strain 15 of the 106 mice treated showed pituitary enlargement with the largest (46 to 87 mg.) consisting largely of adenomas of nonglandular chromophobe cells (33).

Two sarcomas were observed among 16 castrated male mice bearing ovarian grafts (21). There have been many reports of sarcomas developing in mice following the injection of estrogens, usually estrogens in oil (15, 32, 27, 81, 46). In some cases they developed in relation to the oil cysts. Lymphoid leukemia and lymphosarcomas have been observed in a number of strains following the injection of estrogens while none of the controls have shown such tumors (27, 47, 31).

The effect of estrogens in conjunction with carcinogenic agents has been investigated. Reviews of the early studies are available (28, 14).

Hormones and transplantable mouse tumors.—In 1932 Zondeck, Zondek and Hartoch (88) reported an inhibition of growth of the Ehrlich mouse carcinoma following the administration of an extract containing both Prolan A and Prolan B. Using over 400 mice the authors found that the average tumor growth in animals treated over a three week period was 0.2 grams while the control tumors averaged 1.65 grams. Furthermore, it was found that this reduction in growth persisted during later transplants. Many workers have attempted to modify the growth of transplanted sarcomas and carcinomas with pituitary hormones since that time. Some studies confirmed retardation of growth (13, 66, 6). Some reported stimulation (23, 24, 79) and others found no effect upon the growth (36, 39). One of the most serious hazards in such experiments is the difficulty in differentiating the direct and the indirect effects of Prolan on the tumor growth (5). The transplant has been exposed directly to Prolan extract *in vitro* following which it was inoculated into the host. No evidence of inhibition or accel-

eration was secured with mouse sarcoma 180 and slight inhibition of growth was observed with mouse sarcoma S37 (83). Tumor grafts grew more slowly in hypophysectomized animals than in controls of the same age but the relation of final tumor weight to body weight of operated and control mice of equal age was the same (38).

Gonadectomy and sex itself has been considered as a factor effecting the growth of transplantable tumors. Both have been reported of some influence with particular tumors (76, 77, 69, 85).

The extracts of many internal secreting glands in addition to those already mentioned have been used in attempts to alter the growth of spontaneous and transplantable mouse tumors (64, 65, 2, 26, 67, 22, 62, 63). The subject offers interesting possibilities.

Hormone production with tumors.—An adenocarcinoma arising presumably from follicle tissue of the ovary has been tested and found to secrete estrogenic hormone (78). Evidence of estrogenic activity was also seen in one mouse with bilateral granulosal cell tumors (34).

A lengthening and finally cessation of estrous cycles has been noted following transplantation with tumors (60). With the growth of spontaneous mammary gland tumors estrous cycles became infrequent with long continued periods of diestrus, and finally disappeared. Sections of the genital organs showed them to be in extremely atrophic condition approaching that of ovariectomized animals. Cycles were obtained by injection of estrogen. The acyclic condition in tumor mice probably involves primarily the gonadotropic function of the pituitary (1).

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Chapter 9

THE MILK INFLUENCE IN TUMOR FORMATION

By JOHN J. BITTNER, *Roscoe B. Jackson Memorial Laboratory.*

New approaches to the problem of breast cancer etiology in mice developed following the advancement of the extra-chromosomal theory. Independent work was published almost simultaneously by the Staff of the Jackson Memorial Laboratory (28) and Korteweg (20) using strains which had been sent from the Jackson Laboratory. This work has been confirmed in several other experiments with different strains of mice (25–27, 29, 21–23, 19, 5, 8–9, 17–18).

In these experiments reciprocal matings were made between high and low breast tumor strains of inbred mice. When the maternal parents were members of the high tumor strains it was observed that the F_1 and F_2 generation hybrids had a higher breast tumor ratio than if the paternal parents were representatives of the high tumor line. This maternal influence has three possible explanations:

A. That some influence is transmitted in the milk of the potentially breast cancerous females to their young while nursing.

B. That some influence is transferred to the progeny of breast cancerous mothers during uterine development.

C. Cytoplasmic inheritance.

To date all the experimental evidence emphasizes the role played by the milk influence. In all, at least three "influences" must be taken into consideration in the development of breast cancer, at least in mice. These are:

1. A "breast cancerous producing influence" present in the milk of cancer stock mothers.

2. A breast cancer susceptibility due to one or more dominant factors transmitted by breast cancer strain mice.

3. An ovarian or hormonal influence which may or may not be associated with breeding, depending upon the strain of mice studied.

The evidence supporting this explanation follows.

To test the effects of foster nursing on the breast tumor incidence, young born to high breast tumor females were removed soon after birth and were

nursed by females of low tumor strains. The fostered females and their progeny were later used as breeders. The breast tumor incidence in such fostered mice was very low (4, 6-8, 12-18). Similar results have been obtained when females of other high tumor strains were fostered (2, 18).

In later work it was determined that the time interval between birth and the transfer of the young to the foster mother was very important (13). If the young are permitted to nurse their high tumor mother for twenty-four hours or longer there is no reduction in the breast tumor incidence. Progeny of these mice were not observed.

In inbred strains of mice showing a high breast tumor incidence the ratio of this type of cancer is similar among the progeny of the non-cancerous and the cancerous mothers (30, 9). If fostered high tumor females develop breast cancer, the incidence for the first generation progeny is comparable to the control group. With each succeeding generation of progeny there is a decrease in the breast tumor incidence. If the progeny of tested non-breast cancerous fostered females develop mammary cancer, the tendency is not transmitted. An increase in the breast tumor percentage may be obtained by giving the progeny to females of high tumor strains during the nursing period (13).

No significant increase in the breast tumor incidence may be obtained by fostering the young of resistant strain females to high cancer mothers (6, 2, 17). Sub-line differences may account for the variations which have been noticed (3, 1, 17).

The breast tumor incidence in virgin females of high tumor strains depends on the stock. Some stocks have a high virgin incidence (25) while others are very low (11). The foster nursing of young from all types of high tumor strains, tested thus far, resulted in a reduced tumor percentage for females which were used as breeders (3, 18, 2).

If mice of low breast tumor strains are crossed to representatives of high breast tumor stocks, first generation females, used as breeders, which were nursed by females from the high breast tumor line showed a high incidence regardless of the maternal parent. Low ratios were observed in hybrids which had low tumor strain maternal parent and were not fostered, or high tumor maternal parent and were nursed by low tumor strain females. The evidence secured in the reciprocal first and second generation mice is in accord with the theory that breast cancer susceptibility is transmitted as a dominant (8, 17). Foster nursing has no apparent effect on lung cancer development (16).

Additional work with first generation females has demonstrated that the influence usually obtained in the milk may be transferred to some individuals by the inoculation of normal tissue from young potentially cancerous mice (15). This influence may be transferred through the milk to the second generation mice, as expressed by their increased tumor incidence. As stated, females of a resistant strain do not show a high breast tumor ratio if they are nursed by high tumor strain females. Such females, however, receive the "milk influence" which they in turn may pass on by nursing with the subsequent development of breast cancer in animals having the breast cancer constitution.

The nature of the breast cancer producing influence has not been determined. That it occurs in many of the internal organs of high breast cancer strain animals has been demonstrated.

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Chapter 10

INBRED AND HYBRID ANIMALS AND THEIR VALUE IN RESEARCH

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Introduction

During the past few years there has been a tremendous increase in the number of inbred animals, particularly mice, used in research. A large part of this increase can be attributed to the efforts of Dr. C. C. Little, who has not only repeatedly advocated the use of inbred material, e.g. (7), but has, with the aid of students and colleagues, established many inbred strains of mice and made them available in large quantity to other research workers. Thus, the number of mice, mostly from inbred lines, supplied by the Jackson Laboratory to other laboratories has increased from 12,000 in 1933 to 120,000 in 1939.

Nevertheless, any geneticist who samples the recent literature in such fields as physiology, biochemistry, bacteriology, pathology, cancer research, and experimental medicine in general, is struck by three points. First, most of the workers who are still using animals of uncertain origin could profit by the use of inbred stocks. Second, even when inbred animals are used, they are frequently not utilized to their full value. Third, owing to a lack of understanding of the consequences of inbreeding, erroneous conclusions are sometimes drawn from the results obtained with inbred material.

As a geneticist, the author of this chapter may perhaps be permitted to blame geneticists for the above failings. They have provided an excellent theoretical analysis of the Mendelian consequences of inbreeding, and an extensive series of critical experiments that have verified theory and brought new facts to light; but they have expended singularly little effort to sort out and explain those results and conclusions which are of importance to research workers in general. General discussions of inbreeding have been concerned, on the one hand, with the genetic consequences and, on the other, with the relation of these to evolutionary theory, improvement of livestock and domesticated plants, and interpretation of such special phenotypic effects as decline in vigor. Furthermore, of the six recent and better known text-books of genetics only two mention the value of inbred animals in research, and each of these devotes only one paragraph to this topic.

This chapter was planned to bring together and classify those effects of inbreeding which are of general value to experimentalists who are using mice or other laboratory mammals in their research. Much of what is discussed applies, of course, to other organisms as well.

For this purpose the most serious gap in the literature is the lack of an adequate treatment of the phenotypic effects resulting from inbreeding. For example, general discussions of inbreeding have implied, if not definitely stated, that the decrease in genetic variation following inbreeding necessarily results in decreased phenotypic variation. Yet several cases have been reported in which a particular character shows more variation in a certain inbred line than it does in random bred stocks, or did in the stock from which the inbred line was derived. It has been this author's experience that this effect is a seemingly inexplicable paradox to many students and research workers. It has, therefore, seemed desirable to discuss the phenotypic effects of inbreeding in more detail than the title of this book would, at first sight, warrant.

The attempt has been made to present the material of this chapter in a form that can be understood by those not specially trained in genetics.

The few technical terms and simple genetic concepts not explained can be understood by reference to a text-book on the subject.

Genetic Effects of Inbreeding

Following Mendel's work, studies on the mechanism of heredity were naturally focussed on mutations that produced easily recognizable effects. This emphasis on major mutations invited the conclusion that nearly all individuals in any one species have the same genotype (set of genes), the remaining individuals exhibiting mutations. Such is not the case. Genetic studies have shown that in, for example, any wild population of rodents, or any laboratory population not closely selected or inbred, there is tremendous genetic variation; although the population may show none of the major mutations recorded by the geneticist. The changes effected by selection of small phenotypic variations may be cited as one demonstration of this fact.

Before examining the effect of inbreeding on this genetic variation it is necessary to consider how genetic variation is affected by the absence of inbreeding, namely random mating.

Random Mating

Taking the extreme case of an indefinitely large random breeding population, undisturbed by such factors as mutation, it has been shown theoretically that, whatever the original proportions of any two alleles (A, a) may be, the proportions of the heterozygous (Aa) and the two homozygous (AA and aa) classes of zygotes reach an equilibrium in not more than two generations. Further, the relative frequencies of all possible genotypes ($AABbcc$ . . . , $AabbCc$ . . . , etc.) tend to approach an equilibrium in which the different series of genes are combined at random. Linkage has no effect on the ultimate equilibrium. With reversible or irreversible mutations occurring at constant rates there will be an approach to a new equilibrium.

In practice, the above conditions are not found. Such factors as selection and limited size of population will change the relative frequencies of the various genotypes from generation to generation. Provided none of these factors is intensive, however, considerable genetic variation will remain. We can now consider what effect more or less intensive degrees of inbreeding will have on that variation.

Inbreeding

The primary effect of all systems of inbreeding is an increase in the proportion of homozygous gene pairs present in the population. With some

systems, for example brother-sister mating, the population necessarily breaks up into non-interbreeding lines in each of which there is a limited number of parents in each generation. Under such systems an increasing number of genes will become fixed in any one line. Thus, if genes *A* and *a* are both present in the original population, some lines will become fixed so that all individuals in that line are *AA*, other lines will become fixed for *aa*, while others may, in a limited period, not yet have become fixed for that particular gene pair.

This effect of inbreeding is easy to understand for a system as close as brother-sister mating, where, in any one line, there are only two parents for each generation. Merely by chance, matings will occur in which both parents are homozygous for the same gene. Once this has happened all their descendants will be homozygous for that gene so long as they are bred only with each other and no mutation occurs.

The change in proportion of homozygosis with continued self-fertilization was given by Jennings (6). The effects of continued brother-sister mating were investigated by Pearl, Fish, Jennings, and Robbins, and are reviewed by Wright (11). The rate of increase in the proportion of homozygosis, and the limit reached, under systems of less intense inbreeding are by no means easy to see. A general method for determining them has been devised by Wright (11, 17) using his ingenious method of path coefficients. For our purposes it will be sufficient to cite only a few of the results (Fig. 131).

Figure 131 shows that with brother-sister mating (two parents in each generation) the rate of loss of heterozygosis is much more rapid than with double-first-cousin mating (four parents in each generation), although it is considerably slower than that which can be obtained when self-fertilization (one parent in each generation) is possible. The inbred strains of laboratory mammals have been produced almost exclusively by brother-sister mating. With this system, each generation theoretically loses approximately 19% of its heterozygosis in the succeeding generation (except that the fluctuation is wide of this mark in the first three generations). The actual proportions of heterozygosis in succeeding generations, giving the curve in Fig. 131, are: (1, 1/2), 2/4, 3/8, 5/16, 8/32, etc. The proportions can be written for any number of generations simply by following the rule that each numerator is the sum of the two preceding, while the denominators double in each successive generation.

Mating offspring with younger parent, generation after generation, gives the same result as brother-sister mating, with the exception that the average

rate of loss of heterozygosis in sex-linked genes is 29% (50% every two generations) instead of 19%. This system is, then, slightly superior to brother-sister mating. The more frequent use of the latter has probably been dictated by its practical convenience.

It is sometimes required to estimate the percentage of homozygosis in a stock that is inbred, but which has not consistently followed any one

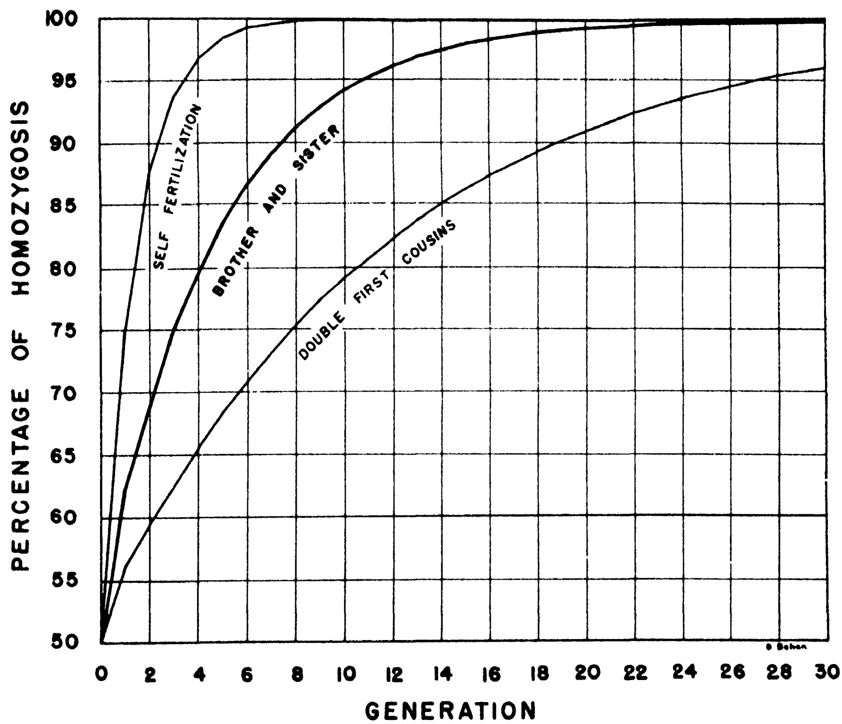


FIG. 131.—The percentage of homozygosis in successive generations under three different systems of inbreeding.

system of inbreeding. If pedigree records have been kept this can be done by the use of coefficients of inbreeding (12, 15, 23).

With brother-sister mating, and any system that results in separate lines of descent, a limit is reached, according to the above calculations, only when complete homozygosis has been attained, that is when all individuals in any one line are genetically identical.

Haldane (5) has discussed various factors that may affect the conclusions reached above. He has shown that linkage may affect the distribution of the heterozygosis left after inbreeding. Thus, the number of organisms in which all the original heterozygosis has been lost may, as a result of

linkage, be considerably higher than would be expected purely from the original number of heterozygous gene pairs. On the other hand, the remaining organisms will carry more heterozygosis than expected. In a similar treatment Bartlett and Haldane (1) have discussed the effects of forced heterozygosis. For brother-sister mating of yellow mice, for example, they have estimated the probability of finding heterozygosis due to linkage with the yellow locus.

A factor that may affect the rate of increase of homozygosis is described by Haldane (5) as follows: "A breeder will probably select the most vigorous individuals as parents. He will eliminate a number of weak or infertile recessives, which will be homozygous for particular genes, and probably so for genes closely linked with them. He may also select for vigor due to heterozygosis as such. Hence at least during the first five to ten generations, when the population is still appreciably heterogeneous, progress towards homozygosis will be slightly slower than the above calculations would suggest."

Haldane gives formulae by which, under various systems of inbreeding, the frequency of heterozygosis, at any locus, due to mutation after inbreeding has begun can be estimated from the mutation rate. Unfortunately, little is known about mutation rate in mammals. Haldane (4) estimates that the gene for haemophilia arises by mutation in the population of London about once in 50,000 life cycles. He concludes (5): "If this is generally true for mammals, and the number of genes is not less than in *Drosophila*, we may expect that as the result of mutation most members of a mammalian pure line will be heterozygous for at least one gene as the result of mutation. Since after 30–40 generations the majority of animals in such a line have lost all their original heterozygosis, the line is then as pure as it is every likely to be."

The last sentence sums up the practical conclusions to be drawn from this section. A later section will show that the genetic uniformity of a given strain in regard to a given character can usually be tested statistically.

Phenotypic Effects of Inbreeding

So far we have considered only the genetic effects of inbreeding. The experimentalist does not work on genotypes, however, he is concerned with characters or phenotypes. It is important, therefore, to consider how the genetic consequences of inbreeding may, in turn, influence phenotypic or character variation. As an introduction to this, it seems desirable to digress briefly on the general causes of phenotypic variation.

General Causes of Phenotypic Variation

The contribution that genetics has made to an understanding of these causes has been brought out clearly by Wright (18). With the aid of diagrams he has emphasized the fact that, however complex the network of processes involved in the development of a character may be, all processes trace back to a gene action somewhere or an external stimulus somewhere.

Wright's general treatment, particularly when applied to mammals, can be extended by distinguishing two main paths by which genes can influence a character. The more direct path is that tracing back from the character to the genes in the individual bearing the character. A character, particularly in mammals, may also be affected by the maternal environment, which,

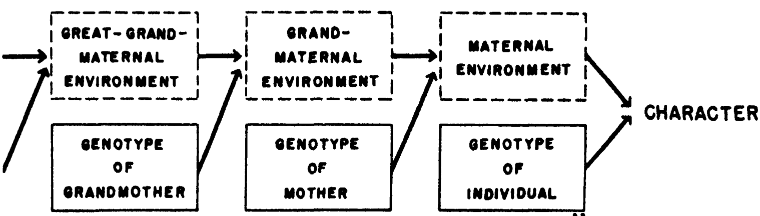


FIG. 132.

in turn, is determined partly by the genetic constitution of the mother. Therefore, a second path traces back through the maternal environment to the genes of the mother. The maternal environment may also be affected by the grand-maternal environment, which, in turn, is determined partly by the genes of the grandmother. The final result of this analysis is an indefinite number of paths, which presumably have less importance the further back they go (Fig. 132).

The genes of the mother, grandmother, etc., are not the only genes external to the individual that can influence a character. Nor is the maternal environment the only medium through which such genes can act. For example, the number of young raised to weaning is determined partly by the genetic constitution of the young, and it has been known to affect not only such characters as gain in weight after birth, but also characters influenced by the temperature of the nest. Variation in tangible environmental factors of this nature can, of course, often be eliminated from an experiment. To make our classification complete, however, the term "biological environment" will be used to group maternal environment with all other environmental factors affected by the genetic constitution of the

stock. There are, then, two main paths by which a character may be influenced by genes: the direct path within the individual, and the path through the "biological environment" from the genetic constitution of the stock (including, conceivably, the genes of the individual), (Fig. 133).

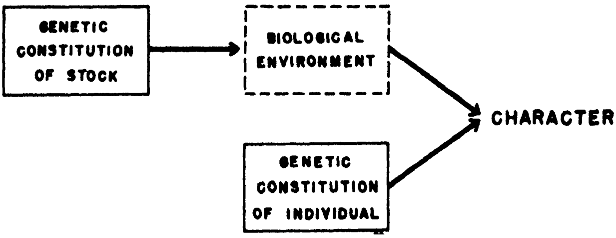


FIG. 133.

To the genetic factors we can now add the remaining cause determining the phenotype, namely the "physical environment," using this term to denote environmental influences which are not affected by the genetic constitution of the stock (Fig. 134).

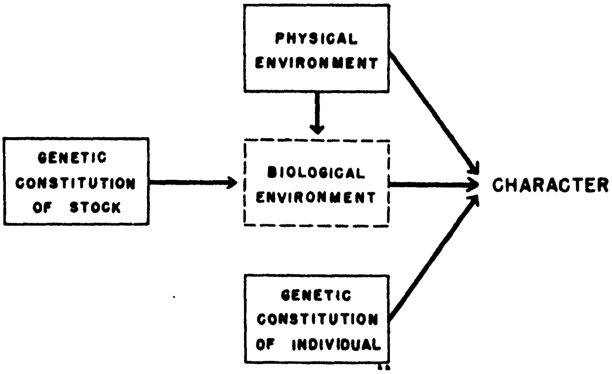


FIG. 134.

The distinction between two major paths of gene action is, perhaps, not essential for an understanding of the rest of this chapter, but it is hoped that it will be of use in emphasizing two points: first, that a character may be influenced by genes other than those of the individual bearing the character; and second, a corollary of the first, that some so-called "environmental" factors may be under genetic control. Since the genetic effects of inbreeding can act on the phenotype through the "biological environment," as well as through the individual, their potential results are greater than is often supposed.

Measurement of Phenotypic Variation

In order to discuss the phenotypic effects of inbreeding it is necessary to express phenotypic variation in terms that the mind can grasp. The two most useful measures of a distribution of biological data are its location and scatter. Thus, the two most significant questions that can be asked about a set of measurements on, for example, tail length in a population of mice are: (*1*) Where is the distribution of lengths located? or: What is the average length? and (*2*) How much spread is there about this average? It will be convenient to refer to these two measures in a general sense as "average" and "variation," remembering that each can be expressed in definite terms by several statistics, of which the mean and standard deviation are respectively the most valuable.

We can now separate the phenotypic effects of inbreeding into effects on the average and effects on the variation. The discussion of some of these will be simplified if we assume that the genetic effects have reached the limiting condition in which there is no genetic variation left, all the individuals in the inbred line having one and the same genotype. Such a limit has actually been reached, at least for all genes with measurable effects on certain characters in certain inbred lines. If, through mutation or insufficient inbreeding, a line is not genetically pure for a certain character, the effects discussed below may still occur, although perhaps not to the full extent possible.

Effect of Inbreeding on the "Average"

The average value of a character will be prescribed by the genotype fixed. It seems extremely likely that this genotype will determine an average that differs from that in the foundation stock from which the inbred line was derived. There will be no change if: (*1*) the character is not affected by the extent of genetic variation possible with the original genes, or (*2*) the effect of the genotype fixed happens to correspond to the average effect of the many genotypes present in the foundation stock. Neither condition can be expected to occur very often. Observation agrees with expectation: a change in average usually occurs with inbreeding and is, as we shall see later, one of the most valuable results for the research worker.

With many characters the change in average may go in either direction. For example, average amount of white spotting in a piebald stock may either increase or decrease with inbreeding. However, with some characters the change is in the same direction in all, or most, of several inbred lines studied.

Thus, average vigor and average fertility usually decline with inbreeding (13). The generally accepted explanation of this phenomenon is that genes unfavorable to vigor, fertility, etc., are usually recessive; and, since inbreeding fixes genes in homozygous condition, irrespective of whether they are dominant or recessive, all the individuals in an inbred line are likely to have a proportion of homozygous recessives higher than that in the average individual in the foundation stock.

Effect of Inbreeding on the Variation

Causes of a change in variation.—It was stated in the introduction to this chapter that inbreeding may lead either to a decrease or to an increase in phenotypic variation. In order to explain both effects it is necessary to distinguish as separate causes: (*1*) the reduction of genetic variation, and (*2*) the characteristics of the resulting fixed genotype. Let us consider each of these in turn.

1. It is apparent from Fig. 134 that reduction in genetic variation will, in itself, tend to decrease variation in a character. In the limiting case, when all genes affecting a character have become fixed, differences between individuals will be determined solely by differences in the "physical environment."

2. The characteristics of the genotype fixed may, however, tend either to decrease, or to increase, phenotypic variation, according as the developmental processes determined by this genotype are less, or more, susceptible to variation in the "physical environment" than are the developmental processes of the bulk of the individuals in the foundation stock.

The total effect of inbreeding on phenotypic variation will be due to a combination of (*1*) and (*2*). If the tendency of (*2*) is either to decrease variation in a character, or not to increase it as much as it is decreased by (*1*), then the character will be less variable in the inbred line. On the other hand, if (*2*) increases the variation more than (*1*) tends to reduce it, then the inbred line will show more variation than the foundation stock.

Here, then, we have an explanation of the paradox, mentioned in the introduction to this chapter, that, although inbreeding causes a reduction in genetic variation, it sometimes results in increased variation in a character. The result is due to the nature of the genotype fixed.*

* From a statistical point of view the paradox can be explained by the type of scale on which the variation is measured. The increase in variation can occur only when the scale is such that the magnitude of the environmental effects differs at different points on the scale. An opportunity is then provided for inbreeding to shift the stock

We can now turn to examples.

Decreased variation following inbreeding.—This is the more commonly observed result. At the moment we need mention only a few examples, such as reduction of variation in: intensity of coat color, amount of white spotting, tissue specificity, and reaction to bacterial inoculation. The degree of reduction differs widely and is dependent on the relative importance of heredity and environment in determining the variation in the foundation stock. Thus, variation in tissue specificity seems to be determined mainly by genetic factors, for it is greatly reduced by inbreeding. In fact, if this character is measured by percentage of "takes" in transplants between individuals, there is commonly no variation left at all in an inbred line, all the transplants being successful. On the other hand, variation in a character like white spotting may be determined largely by the environment and, therefore, not greatly reduced by inbreeding. Wright and Chase (22) measuring white spotting in the guinea pig on an appropriate scale

to a point at which it is more sensitive to the environment than are the bulk of the individuals in the foundation stock. On a scale on which environmental effects are equal at all points, variation cannot increase as a result of inbreeding. A natural scale of this type, with its simple logical relation to the causes of variation, is to be preferred; and when a character does not fall easily into one it is sometimes possible to devise such a scale and transform the data to it (16). If all characters could be expressed in these terms the question of increased variation following inbreeding would not arise. For many characters, however, no such scale has been found, and in some of these there is reason to expect that it would be too complicated for practical purposes. In these cases we can only use the descriptive scales available. It must be remembered that a measure of the variation on these scales, though it may be of descriptive value, does not have the analytical value of statistics derived from data recorded on natural scales.

One of these scales is necessarily quite common in biology because of the frequent occurrence of physiological thresholds in development. On one side of the threshold the character is recorded as "normal," with no variation, while on the other side the character falls into a graded series of "abnormalities." Most of the examples of increased variation following inbreeding given later probably involve physiological thresholds, the random bred stock falling mostly, or entirely, on the "normal" side of the threshold, and the inbred strain falling largely, or completely, on the "abnormal" side.

When a character is recorded only in two categories (e.g., 3-toed and 4-toed, tumorous and non-tumorous, infected and not infected) an inbred strain is to be regarded as more variable than its foundation stock if it falls closer to a 50:50 distribution in the two categories. Here, however, no particular value is obtained by speaking of the "variation," for the distribution of data as recorded can be described completely simply by stating the percentage in either category.

found that the standard deviation of a random bred stock was decreased only 23% by inbreeding.

Increased variation following inbreeding.—The most careful studies of this, as of many other effects of inbreeding, have been made by Wright. To give one example, Wright (19) obtained several inbred strains of guinea pigs that showed more variation than the random bred control stock in respect to number of digits. In one strain, 69% of the animals showed various grades of development of an extra toe on the hind foot; whereas the random bred control stock showed less than 1% with any development of an extra toe. Wright showed that, within each strain, inbreeding had eliminated genetic variation influencing this character. The increased phenotypic variation could be attributed only to fixation of genotypes that resulted in strains more susceptible to environmental variation affecting development of toes.

The Jackson Laboratory C57 black strain of mice shows more variation in development of eyes than that recorded for random bred stocks. In some sublines more than 20% of the females exhibit eye abnormalities ranging from slight cataract to an eyeless condition, although it has been found (unpublished data of the author) that within sublines there is no genetic variation affecting this character.

Green (3) has shown that the Bagg albino strain of mice exhibits a variation in number of presacral vertebrae that is probably greater than that of the original stock prior to inbreeding.

In several strains of mice variation in development of tumors is greater than that characteristic for random bred stocks.

Other cases can be found in the literature. Many more have undoubtedly occurred. The fact that they have not been reported may be due to two causes. First, workers have not been on the look-out for this effect, because most geneticists have stressed the decrease in genetic variation, but have not pointed out how increased phenotypic variation might occur. Second, early work by geneticists was naturally focussed on such characters as intensity of coat color and tissue specificity, that is characters which are not much affected by environmental variation, and which are, therefore, likely to exhibit decreased variation in inbred strains.

Different Effects in the Two Sexes

It should be remembered that even when sex-linked genes have become fixed in an inbred line the sexes will still differ genetically in their sex

chromosome balance. Therefore, the average and variation of a character may, and usually do, differ in the two sexes.

The Value of Inbred Lines in Research

The variety of ways in which inbred lines have already been used in research is extensive enough to warrant an attempt at classification. In the space available here the classification can be illustrated only by a few examples. The two main headings (value of genetic effects and value of phenotypic effects) given below are based on a division of experiments into those in which the emphasis is on the genetic constitution of the stock and those which are concerned with the phenotypic nature of the stock irrespective of its genetic explanation. To date, most of the experiments in the former group have been made by geneticists.

The Value of the Genetic Effects of Inbreeding

Discovering major gene differences in cases obscured by variation in modifiers or environment.—The value of inbreeding, here, lies in the possibility it affords of obtaining at least one of a pair of alleles in homozygous condition along with a uniform set of modifiers. Wright's analysis, reviewed in 1936 (22), of white spotting in the guinea pig provides an excellent example. By crossing self animals with inbred spotted strains and repeatedly backcrossing to the spotted, he was able to show that this character is determined by a major pair of alleles even though it is greatly affected by modifiers and environment.

Estimating the relative importance of heredity and environment.—The importance of environment can be judged by the amount of phenotypic variation remaining in an isogenic inbred line. The importance of heredity in the foundation stock can be estimated from the amount by which variation is reduced by inbreeding. This has already been discussed on p. 335, where it was pointed out that, by this criterion, tissue specificity is determined largely, if not entirely, by genetic factors; while variation in amount of white spotting in piebald guinea pigs is determined largely by environmental factors.

Unless a suitable transformation of scale can be made (see footnote, p. 334) the method is not applicable for an estimate of the importance of heredity when inbreeding results in increased variation. Its use in any case should be guarded by a consideration of the type of scale upon which the character is measured. See Wright and Chase (22) for an example of correct usage.

Estimating the relative importance of various environmental factors.—To gauge the importance of different environmental factors it is naturally desirable to have genetic variation eliminated, or controlled as much as possible. This can be achieved by using inbred strains. Some of the characters studied in this way are: polydactyly (19), and white spotting (22), in the guinea pig; harelip (9), and skeletal variation (3), in the mouse.

Recognizing new mutations.—In mammals particularly, geneticists are anxious to find new major mutations and observe the frequency with which mutations occur. The appearance of a mutant animal is usually recognized more easily in an inbred strain than in a stock in which there is considerable genetic variation.

The Value of the Phenotypic Effects of Inbreeding

Change in average.—This is one of the most used results of inbreeding. An inbred line frequently provides in quantity a type of animal that is rare, or perhaps never observed, in random bred stocks. Thus, inbred lines of mice are available in which the incidence of certain types of tumors is very high. To mention only two uses, these lines are of value both to experimentalists who want spontaneous tumor tissue in quantity and to those who want animals known to be susceptible to tumor growths. Other examples of valuable strains are: those with low resistance to carcinogens and those with high susceptibility to bacterial infection. Thus, the susceptibility of C57 black mice proved of use in testing the protective value of typhoid vaccine (10).

Reduced variation.—Although the preceding effect probably has been used as much as, if not more than, the reduced variation following inbreeding, the reduced variation has been by far the most publicized effect. Its value is obvious when, as frequently occurs, inbred lines are superior to random bred animals in their uniformity of response to such experimental treatments as: hormone injections, feeding deficient diets, administering drugs, excision of organs, exposure to carcinogens, and immunity tests. Results are more clear-cut, and a significant difference between experimental and control can be demonstrated with fewer animals.

We have already mentioned the uniformity of tissue specificity found in inbred lines. This has been of great value in work on transplanted tumors, transplanted normal tissues, and parabionts.

Increased variation.—Increased variation in polydactyly in the guinea pig, and in eye defects and skeletal characters in the mouse, facilitated

studies of the factors affecting these characters; for the amount of variation in random bred stocks was too small for practical analysis.

This effect of inbreeding will undoubtedly be used more frequently when it becomes widely known. Thus, embryologists derive much of their information about normal processes of development from a study of abnormalities, both naturally occurring and experimentally induced. They are already using mutant types and will doubtless appreciate the value of an inbred strain that provides abnormalities covering a wide range.

Uniformity in time.—The genetic make-up of a random bred stock of limited size will drift considerably from generation to generation. Therefore, the characteristics of the stock may differ markedly at different times and thereby cause trouble in a long-time investigation. The genetic constitution of an isogenic inbred line can change only by mutation. The phenotypic nature of the line is, therefore, less likely to vary with time. This applies even when the phenotypic variation is greater in the inbred strain.

Combination of effects.—More than one of the above effects can, of course, often be used in a single investigation. Thus, a strain combining high susceptibility to a bacterial infection with low variation in response to inoculation may be used in a successfully uniform series of tests over a long period.

Differences between inbred lines.—It is to be expected that almost any character studied will be shown to differ in different lines. Differences have already been observed in a great many characters. They cover the range from gene to behavior pattern, including countless biochemical, cytological, histological and gross anatomical characters, and numerous immunological, physiological and embryological processes. We may mention reported differences in calcium content of bones, chiasma frequency in spermatocytes, histology of the adrenal, shape of the xiphoid process, susceptibility to yellow fever, oxygen consumption of excised tissues, development of the mammary gland, and behavior response to a foreign male, as a mere suggestion of the diversity.

The value of inbred lines exhibiting these differences has been shown in many ways. Often they have been used to demonstrate the importance of hereditary factors, or, by crossing strains, to analyse the genetic differences. More frequently they have been employed in studying intermediate causes of a difference, or in searching for associated phenomena to which the difference might be attributed. Thus, Fekete (2) has investigated the histology of the mammary glands in "high" and "low" tumor strains of

mice as a possible clue to the nature of the factors causing mammary cancer.

HYBRIDS

The only type of hybrid that will be discussed here is the one that is of particular value in research, namely the first generation hybrid (F_1) obtained by crossing two inbred strains.

GENETIC CHARACTERISTICS

Apart from the segregation of the X and Y chromosomes, all the germ cells of an isogenic inbred line are genetically identical. It follows that all the offspring obtained by crossing females of one isogenic strain with males of another will have the same genotype. Thus, if one strain is *AABBccdd* . . . and the other *aabbccDD* . . . , all the F_1 hybrids will be *AaBbccDd* . . . Offspring of the reciprocal cross will have the same genotype again in the homogametic sex. Individuals of the heterogametic sex (male, in mammals) will have the same genotype for their autosomes, but a different set of sex-linked genes, their X and Y chromosomes being derived from the opposite parents.

There is one important respect, however, in which hybrids differ from their parental strains: they will not breed true. Though genetically uniform, they are heterozygous for all genes which differ in the two parents. The F_2 will have, therefore, as a result of segregation in the germ cells of the F_1, the maximum genetic variation possible with the genes provided by the parental strains. Backcrosses of the F_1 to either parental strain, and outcrosses of the F_1 to any other strain, will also give considerable genetic variation in the offspring.

PHENOTYPIC CHARACTERISTICS

Average.—The average of a character in the F_1 may fall between the parental averages, it may correspond to either parent, or it may lie beyond the parental values.

Intermediate averages were obtained by Wright (20) in some of his crosses of normal and polydactylous strains of guinea pigs. E. L. Green and the author got similar results in two hybrids from strains of mice differing in number of presacral vertebrae.

The hybrid will, of course, correspond to one of its parents when the character difference is determined by dominant genes all carried by one parent. Thus, the F_1 of a cross between agouti and black strains will be

agouti. There are, however, other cases which do not depend on this simple cause. Wright's thorough analysis of polydactyly in the guinea pig (20) again provides a good example. A cross between a three-toed and a four-toed strain gave all three-toed, yet Wright was able to show that at least four, probably more, genes were involved and that there was no evidence of dominance.

In almost any character connected with vigor or fertility the average in the hybrid commonly exceeds both parents (14), hence the term "hybrid vigor."

Variation.—It is frequently assumed that, because of their comparable genetic uniformity, the parental strains and the F_1 will have the same degree of phenotypic variation. This is a common result, but it is not the only one possible. We have already shown that the extent of the variation in a character in an inbred line is determined by the nature of the genotype fixed, as well as by the absence of genetic variation, for the effect of environmental variation may differ with different genotypes. Similarly, phenotypic variation in a hybrid is dependent on the genotype of the hybrid and may be less or greater than that in the parental strains.

Differences between reciprocal hybrids.—The following three factors may cause a phenotypic difference between reciprocal hybrids. The difference may be one of average, variation or both.

1. The opposite origin of the X and Y chromosomes in the two hybrids may result in a phenotypic difference in the heterogametic sex.

2. The cytoplasm contributed by the mother may differ in reciprocal crosses as a result of gene action in the female germ cells prior to fertilization.

3. The "biological environments" in which the two hybrids develop may differ. In mammals the most important influence of this type is probably the maternal environment. A difference attributable to a maternal effect may have had its origin before fertilization, between fertilization and birth, or even postnatally.

Differences in reciprocal hybrids have been recorded, but the total number is not large. The present author would predict that they will be observed more commonly as geneticists turn their attention away from characters that are not susceptible to environmental variation and hence not subject to the effect of the third factor given above.

The Value of Hybrids in Research

Compared with Inbred Lines

Allowing for the distinction between reciprocal crosses, F_1 hybrids and inbred lines have the same degree of genetic uniformity and comparable

phenotypic characteristics. Therefore, most of the uses which we have listed for inbred strains apply equally well to hybrids. It must be remembered, however, that hybrids will not breed true and that they can be obtained only by maintaining two inbred stocks. In the following respects they are sometimes of more value than inbred strains.

Hybrid Vigor

Some workers who are anxious to eliminate genetic variables from an experiment have, nevertheless, rejected the use of inbred strains because they commonly show decreased vigor. Since hybrids usually combine a high degree of vigor with their genetic uniformity it is surprising that they have not been used more. They are admirably suited for assay tests of hormones and vitamins and for most experiments in which a healthy, vigorous animal is required. The fact that they are commonly highly resistant to disease should be of value to the bacteriologist. For research which requires genetic uniformity, but not the special characteristics of a certain inbred line, hybrids are to be preferred because their vigor makes them more economical to raise.

The Great Variety Available

As the number of inbred strains being maintained increases, the number of hybrids made potentially available increases much more rapidly. Thus, 25 pure strains can produce 300 hybrids (600, if reciprocals are listed separately). There are probably many more than 25 inbred strains of mice available. While many of these have been thoroughly investigated, only a few of their hybrids have been produced and examined. Here, then, is a wealth of genetically uniform material which is almost untapped.

Special Uses

We have already discussed the factors which may cause reciprocal hybrids to differ. The obtaining of reciprocal hybrids is of value when information on the importance of these factors is required. The staff of the Jackson Laboratory (8) reported differences between reciprocal hybrids in mammary tumor incidence in mice. This led Bittner to a discovery of the important post-natal maternal influence on this character (Chap. 9). In collaboration with E. L. Green the author is investigating skeletal differences in reciprocal hybrids obtained in three different crosses between inbred strains of mice. Here, again, it is hoped that the obtaining of a

difference in reciprocal hybrids will lead to increased understanding of variation in the character under investigation.

Another use of hybrids lies in their common ability to grow tumors of both parental strains. This is of value in research and also provides an economical method for maintaining transplantable tumors.

The Building and Maintenance of Inbred Lines

Some of the concepts of value to those workers who wish to start or maintain their own inbred lines are apparent in earlier sections. Others are presented below.

Selection

Inbreeding is often combined with selection for desired characteristics, for example high or low tumor incidence. The effectiveness of various methods of selection on various types of characters is discussed by Wright (21). It may be pointed out here that, when there is a lot of variation which is not genetic, selection of individuals within a single inbred line (e.g., among the offspring of a single brother-sister mating) is of little value. Individuals which are good by accidents of environment, and not by heredity, may be chosen and undesirable genes fixed. In these cases selection is most effective when applied to a number of separate inbred lines; for only between them can real hereditary differences be easily recognized.

Phenotypic variation may remain after a line has become isogenic, but selection cannot change it. In a highly inbred strain selection is, therefore, of value only for its possible control in fixing desirable, or eliminating undesirable, new mutations.

Preserving Vigor

Selection of lines, rather than individuals, applies particularly to vigor and fertility; for Wright (13) has shown that variation in these characters is determined largely by environment. A satisfactory inbred strain can usually be obtained only by starting a large number of strains. In fact, if only one brother-sister line is started from a heterogeneous stock there is a fair chance that it will die out in spite of selection of the two most vigorous animals in each generation.

Tests of Genetic Uniformity

It is often desirable to know whether the variation remaining in a character after considerable inbreeding is due solely to environment or partly to unfixed genes. This can be tested. If there are unfixed genes affecting

the character, then offspring of individuals at one end of the variation should differ from offspring of individuals at the other end. If there is no significant difference in offspring from different types of matings (or if the parent-offspring correlation is not significantly different from zero), then the strain may be assumed to be genetically uniform for the character in question.

Lack of variation in tissue specificity, indicated by 100% "takes" in transplants, is sometimes used as a rough measure of the likelihood of uniformity in genes affecting other characters.

SUBLINES

When a strain is maintained with a large number of animals it should be recognized that, unless matings are made up with reference to a pedigree chart, the strain may break up into many separate lines. These lines may have quite different characteristics if their last common ancestors were not genetically uniform or if different mutations have become fixed.

Among mice supplied to research workers there is, as yet, no universally accepted way of designating the extent of dissimilar ancestry of a given group of animals. One strain may be kept so that all individuals at any one time trace back to a common pair of ancestors as soon as possible. Another strain, perhaps listed under a single name, may have lines of descent which have been separate for many generations. These are often listed as "sub-lines," but this term may be used by one breeder to indicate five generations of separate descent, by another to mean twenty generations, or by yet another only when he has actually observed phenotypic differences between the branches.

The research worker who wants maximum genetic uniformity in his material should, therefore, keep a check on the branching of his own strains and should specify that animals supplied from other sources have a common ancestry within a certain number of generations or exhibit no genetic variation affecting the character under investigation.

RISK OF CONTAMINATION

Since a high degree of homozygosis is obtained only after many generations of inbreeding, a single unfortunate outcross may undo years of work. In a mouse colony in which different lines are maintained an accidental outcross may occur as a result of faulty pens, into which stray animals can enter, or to the returning of animals to the wrong pen after removal for any purpose. Risk of the latter can be reduced to a minimum by handling different lines and sublines at different times and by keeping them in

separate parts of the laboratory or cage rack, certainly not in adjacent sections of wooden boxes. When several inbred lines are to be started it is desirable to mark them with different coat colors or other genetic characteristics contamination of which will be readily recognized.

Effect of Relaxing Inbreeding

If a strain is to be maintained with maximum homozygosis, there should be no relaxation of inbreeding. Relaxation of inbreeding in a population containing different sublines would, of course, introduce heterozygosis immediately. It should be avoided even in an isogenic line; for it would tend to preserve heterozygosis introduced by mutation. Haldane (5) has discussed this effect.

There is one case, however, in which relaxation of inbreeding might be of advantage. If a large group of animals is to be set aside for experimental purposes, it is preferable to set aside a single pair and breed their descendants at random to obtain the experimental animals. The effect of this is to distribute at random throughout the population any genes which are unfixed in the pair set aside. In practice, however, it is usually adequate to take all the animals from the inbred strain provided they have a recent common ancestry.

Fallacies

Misinterpretation of Variation within Strains

Publicity on the genetic uniformity to be obtained by inbreeding has apparently led some experimentalists to expect complete phenotypic uniformity; although their own observations on variation in such characters as litter size, and weight at weaning, must belie this conclusion. Possibly the extreme uniformity obtained in a few characters like tissue specificity, and coat color, is responsible for this view. At any rate, surprise is sometimes expressed when a character is found to show variation in an inbred strain, and attempts have been made to explain the variation away, particularly when it shows itself in an all-or-none effect, such as tumor or no tumor. Thus, the fact that some tumors occur in a "low" tumor strain has been attributed to residual heterozygosis, and the occurrence of non-tumorous animals in a "high" tumor strain has been "explained" by stating that these animals would have had tumors if they had lived long enough. These explanations may be true in special cases, but the former cannot apply when the homozygosis has been tested, and the latter will not account for variation in time of appearance, rate of growth, region affected, etc.

It is hoped that the earlier part of this chapter has made it abundantly clear that phenotypic variation is usually present in an inbred line as a result of environmental causes and that, although it is usually less, it is sometimes actually greater than that in random bred stocks.

It might be thought that this variation could be reduced by giving more attention to the uniformity of the laboratory environment. This would have little effect, however, on the many characters whose variation is due largely to intangible factors in the maternal environment. Wright's elaborate search for the environmental causes of variation in white spotting in the guinea pig (22) ended with 89% of the variability due to causes which he could classify only as "developmental accidents." Wright has suggested that the degree of irregular asymmetry in the expression of a character serves as a rough estimate of the importance of these factors and, therefore, of the extent of variation to be expected after inbreeding.

Misinterpretation of Differences between Strains

There is a common belief that a character occurring only in alternate categories (as opposed to the other extreme: a continuous distribution) must be due to alternate genes in the same way that agouti and black coat color are. This has sometimes led to a hunt for a single major gene difference as the cause of a character difference that shows no, or little, overlap in two inbred strains. Thus, attempts have been made to find a single pair of alleles responsible for the difference between "high" and "low" tumor strains. Such a hunt is all right if it is critical. It can only be critical if cognizance is paid to the fact that, owing to the common occurrence of biological thresholds, of all-or-none processes in development, many characters are necessarily alternate in expression. Many genes may be involved, the effects of some combinations falling below the threshold, while the effects of the others fall above. If this fact is realized it will be appreciated that apparent dominance in the F_1 of a cross between strains, a 3:1 ratio in the F_2, and a 1:1 ratio in the backcross, are not critical criteria of the presence of a single major pair of genes. Many genes may be involved and the above generations happen to be cut by a threshold of effect into approximately the above proportions. In one of his crosses between three-toed and four-toed strains of guinea pigs, Wright (20) actually obtained the above ratios, but was able to show that at least four factors were involved. The critical experiment is to test the genetic nature of the types apparently segregating in the backcross or F_2 by breeding them with the "recessive" stock.

It should also be borne in mind that some differences that have been attributed to genetic causes may be due to parasites. Because of the limited number of parents, there is a relatively high probability that an inbred line will become uniformly infected, particularly with parasitic organisms that are transmitted from mother to offspring. It is not impossible that some mammary cancer differences between strains may be due partly to this cause.

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Chapter 11

PARASITES

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INTRODUCTION

The house mouse has not been slighted by the evolutionary processes developing parasitic forms. Over a dozen protozoa have been described as parasites or commensals living in its blood stream, digestive tract, and various other internal organs. Of the nematodes, Hall (17) lists twelve species for which the mouse may act as host, and describes from the rat a thirteenth which other workers have found in the mouse. A number of species of tapeworm infest it, the adult forms of some living in its digestive tract and the larvae of others in its tissues. Also, to this group of internal forms might be added the mites, lice, bedbugs, and fleas which may occur as external parasites.

Many of these parasites are of utmost importance to the research worker who is employing mice in his experiments. Although probably comparatively few of the forms have much influence on the well-being of the mouse in the natural state, under laboratory conditions and especially under experimental conditions they may develop into serious factors not only because of their deleterious effects upon the mouse, but also because they may act as influencing factors introduced into the experiments. Animals used in testing deficient diets may have their resistance so lowered that external or even internal parasites may get out of control. The death of a mouse bearing a large tumor may be affected not merely because of the large

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tumor but because with the tumor the animal was unable to keep his parasites in check. Deviations from the normal blood count may be due to parasites rather than to the experimental factors under consideration.

There is not only the havoc caused directly by the parasites to be guarded against, but also that which they may cause more indirectly by acting as transmitters for pathogenic viruses, bacteria, and protozoa. Although not so many mouse diseases have been proved to be spread in such manner, it seems possible that especially the blood-sucking parasites—bedbugs, mites, lice, and even fleas are of much greater importance as vectors than is definitely known.

Sometimes the fact that the mouse may act as host for so many parasites proves to be an advantage for the research worker. In many instances it has afforded a convenient way to study phenomena of parasitism. Basic principles discovered in studying mouse parasites can well be applied to parasites of man or domestic animals which do not lend themselves so readily to experimentation. Also, some forms pathogenic to man or domestic animals may be caused to take up their abode in the mouse. Thus, a very convenient living culture chamber is provided for the parasitologist.

In this chapter an attempt has been made to discuss briefly many of the protozoon, helminth, and arthropod parasites which may be expected to be found infesting laboratory mice.

PROTOZOA

AMOEBAE

Endamoeba muris (GRASSI, 1879).—This (Fig. 135) is probably the most common amoeba found in the mouse. Of 85 house mice collected at Durham, N.C., Harkema (18) found as many as 13.09 per cent harboring this protozoan in the small intestine. It has also been reported as found in the caecum and colon of mice and of rats.

Structurally *E. muris* is very similar to *E. coli*. Tryphozoites will average 30 μ in diameter. They display protruding pseudopodia with glassy covered ectoplasm and a fine granular zone. In the cytoplasm are occasionally lustrous granules. The nuclear membrane is thick with peripheral chromatin granules, and there is a large karyosome. Coccal bacteria may be enclosed in the cytoplasm. Cysts measure 15 μ–20 μ in diameter, and when mature they contain eight nuclei although two and four nucleated cysts may be found.

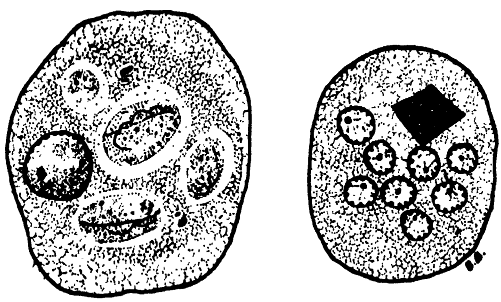
E. muris is probably also much like *E. coli* in that it is not pathogenic but lives more as a commensal in the lumen of the intestine of the mouse.

Walker (42) describes three other amoebae from the intestinal tract of the mouse, namely: *Amoeba enterica, A. musculi*, and *A. fecalis*.

FLAGELLATES

Trichomonas muris (GRASSI, 1879).—*T. muris* may well be classed as one of the most common intestinal protozoa of the house mouse. In the above mentioned work of Harkema it was found in the caecum of 30.95% of the mice examined.

Wenyon (43) describes *T. muris* in the mouse as a pear-shaped organism in the trophozoite stage varying from 3 μ to 20 μ in length (Fig. 136). There



A. B.

FIG. 135.—*Endamoeba muris* from the rat (×1500). A, Trophozoite; B, cyst. (*After Wenrich.*)

are three anterior flagella and an undulating membrane bordered by an axoneme which continues as a posterior flagellum. In the anterior region are located the oval nucleus, a slitlike cytosome, and two groups of closely aggregated blepharoplasts. The flagella arise from the most anterior one of the blepharoplasts, and posteriorly from them extends the axostyle which terminally protrudes as a short point. Food vacuoles containing bacteria are found in the cytoplasm. Reproduction is by longitudinal fission or multiple segmentation.

Cysts of *T. muris* have been described by Wenyon as about 6 μ to 8 μ in diameter. He stated that it is difficult to judge whether or not the organism is encysted since the flagellates may become spherical and quiescent in passed feces although not forming a cyst.

Although trichomonads cause disorders or have been accused of causing disturbances in man and some lower animals, no pathological condition has yet been attributed to *T. muris*.

Hexamita muris (GRASSI).—This (Fig. 136) is also a rather common flagellate which occurs in the intestine of mice and rats. It possesses six

anterior flagella and two posterior flagella which arise from axonemes. There are two nuclei situated near the anterior end. Wenyon (43) describes the trophozoite in the intestine as being from 4 μ to 7 μ in length, while a larger form occurring in the caecum measures as much as 10 μ. The cysts are oblong, measuring 6 μ to 7 μ in length and 3 μ to 4 μ in breadth. In the cysts nuclear division occurs, and multiplication is also by longitudinal fission of the trophozoite.

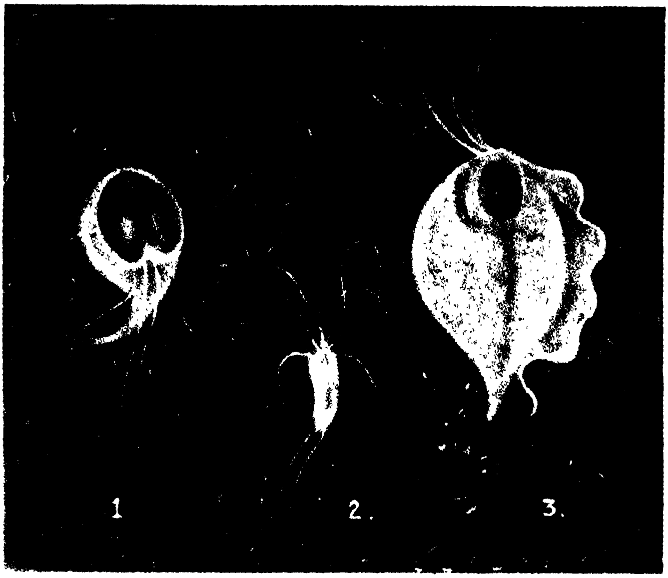


FIG. 136.—Flagellates from the intestine of the rat as seen when alive. 1, *Giardia muris;* 2, *Hexamita muris;* 3, *Trichomonas muris.* (*After Hegner.*)

Giardia muris (GRASSI, 1879).—According to Hegner (20) *G. muris* occurs in a considerable portion of laboratory rats and mice. The trophozoites live in the small intestine while the cysts can be found in the caecum and the colon or in the feces (Fig. 136).

The trophozoite is a flattened, pear-shaped, bilaterally symmetrical organism measuring on the average 9.8 μ in length and 6.75 μ in breadth. A large ventral anterior sucker attaches it to the intestinal epithelium. Two nuclei and a pair of blepharoplasts are located in the anterior region. From the blepharoplasts arises a pair of flagella which pass anteriorly and after crossing near the extreme anterior margin pass laterally to emerge one on either side of the organism. A second pair of flagella which also arise from the blepharoplasts pass posteriorly to emerge laterally toward the posterior end of the body. A pair of axostyles (single, according to Kofoid and

Christiansen (25)) connect the blepharoplasts with the posterior tip of the organism where they give rise to the pair of caudal flagella. A fourth pair of flagella arises ventrally from the axostyles just posterior to the nuclei. Two deeply staining bodies lie dorsal to the axostyles. Both binary and multiple fission take place in the nonencysted stage (19).

Cysts form and are passed out with the feces, and undoubtedly infection occurs by the ingestion of the cysts in contaminated food and water.

Slight infections are apparently not greatly harmful to laboratory mice, although more severe infections cause enteritis. Kofoid and Christiansen (25) have noted that in mice the organism gives rise to a readily recognizable enteritis which appears as a chronic condition in young mice.

HAEMOFLAGELLATES

Trypanosoma duttoni THIROUX.—The trypanosome described as occurring naturally in the blood plasma of the mouse is *T. duttoni*.

As is typical of trypanosomes this form is a spindle-shaped organism. The flagellum arises near the posterior end of the body, passes anteriorly, is connected to the body by an undulating membrane, and extends beyond the anterior end. This species of trypanosome is quite slender, measuring about 25 μ in length, and the flagellum is long. Anatomically it cannot be distinguished from the more familiar *T. lewisi* found in the rat.

Apparently the life cycles of the two forms are also much the same. It is well known that *T. lewisi* employs the rat flea as its intermediate host, the rat becoming infected by swallowing the feces of the infected flea; and Brumpt (6) has shown that the swallow flea could be made to act as the intermediate host for *T. duttoni*. He demonstrated a cycle development in the swallow flea and was able to infect mice by feeding them feces of the infected fleas. While this was obviously not the natural intermediate host, it does suggest that fleas occurring on mice may well act as the vectors.

Trypanosoma duttoni like *T. lewisi* is generally considered to be nonpathogenic, but obviously it can occasionally cause fatal infection. Roudsky (32), by rapid inoculations from rat to rat of the whole blood of an animal when the trypanosomes were at the multiplication phase, was able to raise the virulence of *T. lewisi* until it was not only transmissible to the mouse but was definitely pathogenic for the mouse as shown by the hepatic and splenic lesions caused, and the infection proved to be transmissible from mouse to mouse. Later (33) he was able by a similar procedure to increase the virulence of *T. duttoni* until it was infective when inoculated into the rat.

COCCIDIA

Eimeria falciformis (EIMER).—Mice are commonly infected with *E. falciformis* (Fig. 137). Although it has been listed as a coccidium of the rat (22), several workers (43) have shown that it cannot be transmitted to the rat nor can mice be infected with the rat coccidium, *E. miyairii.*

Development involves the schizogony and the sporogony cycles. Infection occurs by the ingestion of the mature oocysts, each of which gives rise to eight sporozoites which enter the epithelial cells of the digestive tract and there undergo schizogony. This occurs chiefly in the small intestine but may also occur in the large intestine (43) or in the stomach (31). The merozoites liberated from the schizonts may repeat the asexual cycle, or they may develop into microgametes and macrogametes. Fertilization results in the formation of a zygote which later develops into the oocyst. These occur in the feces and can readily serve for diagnosis. They are subspherical and measure 16 μ to 21 μ by 11 μ to 17 μ.

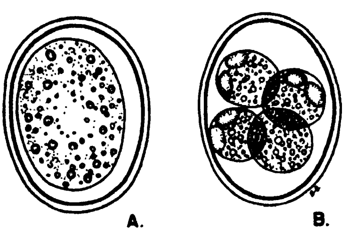


FIG. 137.—Stages in the development of the oocyst of *Eimeria falciformis* (×1000). (*From Wenyon.*)

It is known that laboratory mice often may carry a mild infection of *E. falciformis.* Yet, Nieschulz and Bos (27) in studying the course of infection found that mice free themselves of an infection with *E. falciformis* within 26 days when prevented from acquiring reinfection. They suggest that the chronic condition of spontaneous infection is probably due to reinfection.

E. falciformis is pathogenic, although in mild infections the hosts are not injured very severely. Of 50 mice which Nieschulz and Bos experimentally infected with the coccidium, 40 per cent died between the fourth and eighth day after the infection. They reported that the death was due chiefly to the breaking down of the intestinal epithelium by the schiozogony forms. Wenyon (43) states that in acute infections the organisms may cause acute enteritis.

Cryptosporidium muris TYZZER, 1907; **Cryptosporidium parvum** TYZZER, 1912.—Two other coccidia of the digestive tract of the house mouse are *Cryptosporidium muris* Tyzzer (40) and a smaller species, *C. parvum* Tyzzer (41). *C. muris* is found in the stomach while *C. parvum* lives in the small intestine. They differ from *Eimeria falciformis* in that neither is intra-

cellular, *C. muris* being strictly extracellular, while *C. parvum* might be classed as intermediate.

C. muris lives in the gastric glands. During growth the forms occur on the surface of the glandular epithelium, and all forms except the merozoites and sporozoites possess a limiting membrane and an organ by which they are attached to the surface of the epithelium. The schizonts, which reach a maximum size of 7 μ by 6 μ, give rise to eight merozoites. The mature oocyst is approximately 7 μ by 5 μ, and it becomes a single spore containing four sporozoites. Many of the sporozoites are set free before passing from the stomach, and Tyzzer suggests that probably autoinfection may be affected through sexual as well as through asexual reproduction.

C. parvum develops in the cuticula of the epithelium of the intestinal villi. The forms at first bury themselves in this layer, becoming attached by an attachment organ to the membrane limiting the cuticula from the cytoplasm. As they grow they protrude from the free surface of the cuticula, but they never penetrate the cytoplasm. Like *C. muris*, eight merozoites are produced by each schizont and four sporozoites by each oocyst. The maximum diameter of the schizonts is 5 μ, and the mature oocysts do not exceed 4.5 μ.

Evidently both species are quite commonly and widely distributed among laboratory mice. However, neither is of great importance pathologically, although Tyzzer reports that in extensive infections *C. muris* does cause dilation of the gastric glands and some leucocytic infiltration of the gastric mucosa. No inflammatory processes are reported resulting from infection with *C. parvum*.

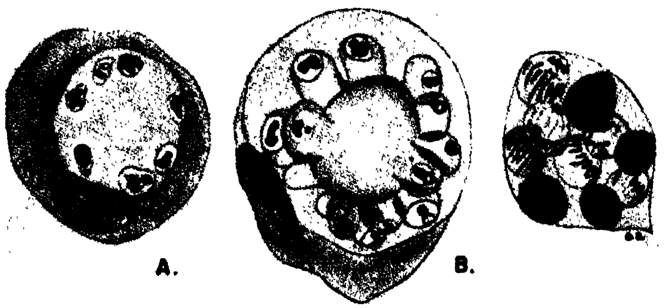
Klossiella muris SMITH AND JOHNSON, 1902.—This sporozoan, which Kudo (26) considers under the order Coccidia, infects the kidneys of mice. It was first discovered by Smith and Johnson in 1889, and later they made a study of it from the kidneys of adult gray mice caught in the animal room of the laboratory at Harvard University (36). They describe the external appearance of the kidneys as being slightly enlarged with a very delicate mottling of the whole surface by minute, barely visible, grayish specks.

European authors (22, 43) have since described it as a common parasite of white mice. Cannarella (8) encountered it in his mice used for tumor studies. In 33 mice of an experiment with artificially grafted tumors he found the organism infecting the kidneys of 43.7% of the mice with a tumor and 40% of the mice lacking tumors.

The schizogony cycle takes place in the endothelial cells of the capillaries of the glomeruli. Young gametocytes produced by this asexual cycle make

their way into the urinary tubules, and the sexual cycle occurs in the epithelial cells of the convoluted tubules. Sporogony stages are shown in Fig. 138. The sporocysts pass down the tubules and escape with the urine. Infection can be affected by the administration through the mouth of urine from an infected mouse.

According to Jaffé (22) the parasite may also be found in the capillary endothelial cells of the lungs and spleen. He points out that "infiltrates" found in the cells of the kidney and especially of the lungs "suggest pathogenic qualities." Cannarella explains the interstitial infiltration as the result of mechanical action and alteration of materials by *K. muris*. He



A. B. C.

FIG. 138.—Sporozony stages of *Klossiella muris* within kidney cells. A, mother-sporoblast (×1435); B, daughter-sporoblasts (×1590); C, spores (×870). *(From Smith and Johnson.)*

finds that occasionally the interstitial infiltration leads to a sclerosis of the organ accompanied by the reduction of the functional parenchyma. The minute specks mentioned above which are seen externally represent necrobiotic changes in the cortex. J. M. Twort and C. C. Twort (39) state that undoubtedly in most cases this organism is the cause of nephritis in the mouse, and that they expect to find *Klossiella* nephritis in at least 90 per cent of their animals after they have reached the age of 12 months. This was concluded after about 12,000 post-mortem examinations. Other organs where they have found the parasite include the brain, suprarenal, lung, thyroid, spleen, lymph glands, and pituitary.

This organism represents an excellent example of a parasite which may introduce confusing factors into an experiment. Cannarella well recognizes this for he writes: "Il est donc indispensable que les rechercheurs et les

expérimentateurs connaissent parfaitement les altérations de dégéné réscence et d'infiltration qu'on rencontre constamment dans les reins atteints de coccidiose, afin de ne pas mettre en relation ces phénomènes avec d'autres causes étrangères au *coccidium* qui n'est pas toujours bien reconnaissable, qui n'est pas toujours reconnu et qui, pour le passé, a constitué souvent une cause d'erreur." This statement could well be broadened to include the other organs infected by the parasite.

SARCOSPORIDIA

Sarcocystis muris BLANCHARD.—This was the first species of *Sarcocystis* to be described, having been discovered by Meischer in 1843, infecting the muscle of mice. Since that time it has been found in the rat, and other species have been found infecting various other animals. However, the most extensive studies have been with *S. muris* since its hosts can be easily infected by feeding them infected tissue.

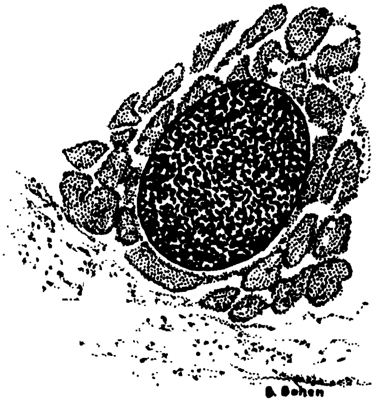


FIG. 139.—*Sarcocystis muris* embedded in the striated muscle of the mouse. Cross section of a "Meischer's tube." (×75.)

These parasites (Fig. 139) can be seen as tiny white streaks known as "Meischer's tubes" imbedded in striated muscle tissue or less commonly in non-striated muscle. The tubes may be as much as 5 cm. long or they may be so small as to be seen only with the microscope. They are filled with sickle-shaped spores called "Rainey's corpuscles." When the spores are ingested by the host a small amoeboid body is liberated which penetrates the epithelial cells of the intestine. Here schizogony occurs producing merozoites which make their way to the muscle tissue where after about forty days multinucleated plasmodia can be found. Cells may be liberated and reinfect other muscle fibers until an intense infection is reached. Ultimately development progresses to form the Meischer's tubes containing the spores.

In some cases no serious results are apparent with the infection, although death of mice occurs with heavy infection. It has been shown that Sarcosporidia produce a toxic substance, and this is probably responsible for the death of the host. For a more detailed discussion see Wenyon (43).

Prevention and Control of Protozoan Infections

When dealing with laboratory mice, prevention and control measures are of much greater importance than treatment of protozoan infections, since the life span of the mouse is so short and new animals can be so quickly produced. However, there is no reason that some of the treatments recommended for the different protozoan diseases of higher animals could not be employed with some success providing they were regulated to suit the smaller animal.

With most of the intestinal protozoa, infection occurs by ingesting the cyst forms which have passed out of the body in the feces. Therefore, control measures should be directed toward preventing the contamination of the food and water. A feeding and watering arrangement such as is described by Bittner in the chapter on Care and Recording is excellent for this reason. There is absolutely no way in which the water can become contaminated, and there is only a slight chance of the feces coming in contact with the food. It is inadvisable to feed and water laboratory mice in open containers placed on the floor of the cages for such practice lends itself perfectly toward the perpetuation and spread of intestinal protozoan diseases. Mouse food should be stored in mouse-tight containers and feed rooms to prevent it from being overrun by stray mice which may be infected. Insect pests such as silver-fish and cockroaches may be the means by which the mouse food becomes contaminated. Thus, they should be eradicated.

General hygienic procedures in caring for the cages are essential in preventing intestinal protozoan epidemics. The cages should be cleaned frequently and well bedded, not only to keep the mice from trampling over the fecal material, but also to keep the cages dry. Most protozoan cysts require moisture to live. The use of ordinary disinfectants in cages is not generally effective in destroying the cysts of various protozoa. The use of live steam on the cages or the emersion of the cages in a steam bath is recommended for the destruction of cystic forms.

The above control measures would also apply to the kidney coccidium, *Klossiella muris*. In this case infection occurs when the food and water have become contaminated with the urine of the infected mouse.

In cases in which the parasite requires an intermediate host to complete its life cycle, control measures can be most effectively directed toward the elimination of the intermediate host. *Trypanosoma duttoni* is suspected of employing the flea as an intermediate host, and elimination of the flea would probably be the easiest method of controlling the protozoan.

If Sarcosporidia appear in laboratory mice, the infection can be prevented from spreading by barring any cannibalism on the part of the mice. The spores are imbedded in the muscle tissue, and they give rise to infection when ingested by a susceptible host.

HELMINTHS

TREMATODA

Although there can be found in the literature descriptions of trematodes occurring in the house mouse, *Mus musculus*, it would seem highly improbable that any would be found infesting laboratory mice, in view of the fact that they commonly employ some species of snail in which to complete their life cycles. However, laboratory mice can be infested with certain species of trematodes, thus, in some instances supplying convenient aids in life cycle studies. Such an example is reported by Price (30).

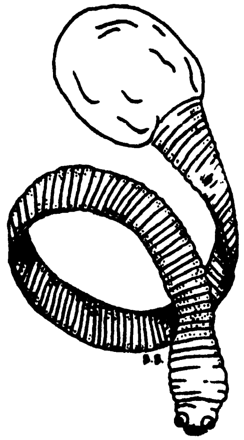


FIG. 140.—Strobilocercus of *Taenia taeniaeformis*. (*From Augustine.*)

In her life cycle studies of the blood fluke *Schistosomatium douthitti* (Cort), Price found that the adult would thrive in *Mus musculus* although she considered *Microtus pennsylvanicus* as the natural host. In her experiments in which *M. musculus* was employed, the life cycle of the parasite was revealed. It was found that the adults of the species live in the hepatic portal veins of the mouse. The gravid females make their way to the small veins of the intestinal wall and there deposit their eggs. The eggs rupture through the lining of the intestine and pass to the exterior with the feces. After development in the snail, the cercariae reinfect mice by penetrating the skin of the host and passing along the blood stream to the hepatic portal veins where they reach maturity.

CESTODA

Probably the most important of the worms which may parasitize laboratory mice are the tapeworms. Some live in the mouse as adults infecting the intestine or bile duct, while others employ the mouse as an intermediate host and live in the liver or mesenteries. Stiles and Hassall (38) list for the house mouse as many as 14 different species, obviously some of which occur so rarely as to be of little importance. Five species including the more common and more interesting ones are discussed here.

Taenia taeniaeformis (BATSCH, 1786).—Also known as *T. crassicollis*. This is a tapeworm which commonly employs the mouse or rat as an intermediate host. The larval stage, which bears the name *Cysticercus fasciolaris*, is a strobilocercus (Fig. 140). It develops within a cyst in the liver of the mouse or rat. The adult is a very common tapeworm of the intestine of the domestic cat having also been reported from wild cats (Fig. 141).

Infection in mice is effected by ingesting the eggs. In the intestine the shells are digested off, liberating the onchospheres which penetrate the wall of the intestine and make their way to the liver via the hepatic portal system. On the second day after ingestion of the egg the onchosphere has reached the capillaries of the liver. A cyst wall forms around the larva which develops into the strobilocercus lying free within the cyst bathed in a clear, yellowish fluid. Cats become infected by ingesting infected mouse or rat livers.

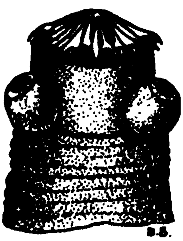


FIG. 141.—Scolex of *Taenia taeniaeformis* (×15). (*From Hall, after Neuman.*)

This is an especially interesting parasite in that from the walls of the cysts sarcomata of the liver develop. Bullock and Curtis (7) in 1920 reported producing cysticercus tumors in some 201 rats by feeding them the eggs of the parasite. Later it was used extensively in tumor experiments, especially by Dunning and Curtis (12).

Except when being used in tumor studies, this parasite quite obviously would be very undesirable in laboratory mice. However, control measures are not difficult, for if the primary hosts, i.e., cats, are eliminated from the laboratory, the main source of infection is removed. If it is desirable to have a cat around the building, as sometimes one proves quite indispensable in keeping down wild mice, the cat should not have access to the feed room or be permitted to climb over the feed or bedding at any time. Periodic examination and treatment of infected cats constitutes a control measure of value for preventing infection in mice.

Taenia pisiformis (BLOCH, 1780).—This is a common tapeworm of dogs and is occasionally found in cats. The larva is a cysticercus (*Cysticercus pisiformis*) which develops in the liver and mesenteries of the rabbit and has been reported from the mouse. However, the paucity of the records of the cysticercus occurring in the mouse minimizes the prospects of its becoming a pest among laboratory mice. For a rather complete account of its development see Hegner, Root, Augustine, and Huff, Parasitology, page 318 (20).

Hymenolepis fraterna (STILES, 1906).—Many authors apply the name *H. fraterna* to the common "dwarf tapeworm" of the mouse and rat, thus

separating it from the form found in man. However, others feel that the evidences are not sufficient for separating the rodent form and the human form into two distinct species and apply the earlier name *Hymenolepis nana* (von Siebold, 1852) to the forms found in mouse, rat, and man. Morphologically the forms are identical and their life cycles are the same. Also, the rodent form and the human form are interchangeable, although in some experiments they have not developed as readily in the alternate host as they did in the host in which the parents developed. However, Shorb (35) has shown a difference between the rat form and that found in the mouse. He has found that although strains from wild rats are equally infective for rats and mice, strains from mice are more infective for mice than for rats. Thus, it would seem that while the parasites probably originated from one form, which Augustine considers to have been that of the mouse (20), there have since developed definite differences in the three forms.

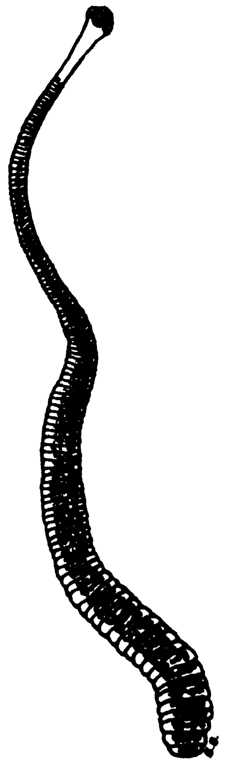


FIG. 142.—Adult dwarf tapeworm, *Hymenolepis nana*. (Enlarged.) (*From Stiles and Crane, after Leuckart.*)

The adult worm is quite small (Fig. 142). Measurements given by Augustine (20) are: length 10 mm. to 45 mm.; breadth 0.5 mm. to 0.7 mm.; diameter of scolex 0.25 mm.; and length of hooks 14 μ to 18 μ. The scolex is globular, and on the rostellum the hooks form a single row. They number from twenty-four to thirty. The strobila may contain as many as 200 proglottids. The eggs (Fig. 143A), which usually occur in large numbers in the feces of infected animals, measure from 40 μ to 60 μ in diameter. There are two membranes, the inner of which gives rise to filiform projections at each pole.

No intermediate host is required for development. Grassi (1887) proved that infection occurred by the ingestion of the eggs which are infective immediately after they have passed out of the host. Upon entering the intestine the eggs give rise to cysticerci which develop in the villi of the small intestine, usually limiting themselves to the anterior one-half (Fig. 144). Later the cysticerci produce adults which become attached to the epithelium toward the posterior part of the small intestine.

Although probably quite unusual, an intermediate host may be employed in the life cycle. Bacigalupo (3) has shown that when eggs are ingested

by certain insects including the adult *Tenebrio molitor* and *T. obscurus*, cysticerci will develop which in turn grow into adult worms when the infected insect is eaten by the primary host.

A third possible way of infection is by the development of worms within the intestine from eggs that have never passed out of the host, i.e., internal autoinfection. However, Hunninen (21) has shown that this does not occur in normal mice, for which he suggests two reasons: first, that the cysticerci develop anteriorly to the region where the adults are found, and second, that from 5 to 18 days after the first infection there is an absolute resistance to

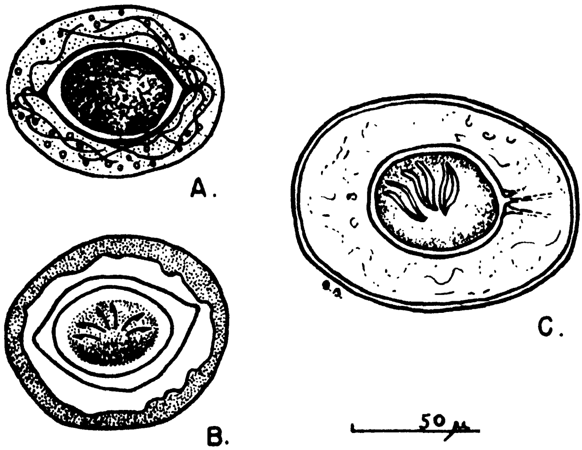


FIG. 143.—Eggs of Cestodes found in the mouse. A, *Hymenolepis nana* (*from Augustine*); B, *H. diminuta* (*from Augustine*); C, *H. microstoma* (*from Joyeux and Kobozieff*).

further infection. He does suggest, however, that autoinfection may occur in mice whose resistance is lowered as with a bacterial infection.

Treatment of mice infected with *H. fraterna* is hardly feasible, which is generally true with mice parasitized by helminths. Instead, it would seem more advisable to make the attack with preventive and control methods. With *H. fraterna* the fact that internal autoinfection does not normally occur simplifies control measures for laboratory mice. Little difficulty should be experienced with the parasite if water and food is kept so that it cannot be contaminated with feces and if the cages are regularly cleaned. Keeping the cages clean will also help to eliminate some of the insects which may act as intermediate hosts.

That the tapeworm may be transferred from the mouse to man makes it important for one working with mice to take precaution against becoming infected. One should form the habit of washing his hands each time he has

finished handling mice lest his hands may have become contaminated with the eggs which might be transferred to the mouth.

Hymenolepis diminuta (RUDOLPHI, 1819).—This (Figs. 143B, 145 and 146) is one of the most common tapeworms of the mouse and rat, and it sometimes occurs in man. It is also cosmopolitan in its distribution, having been reported from various places in the United States, Europe, and South America. Bacigalupo (2) reports that 28 per cent of 300 rats from Buenos Aires were infected.

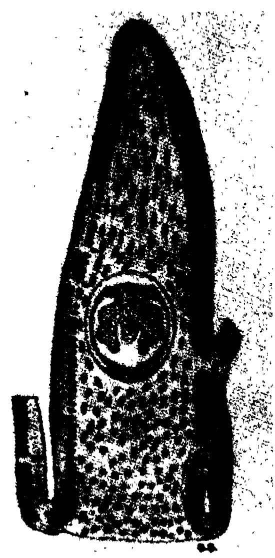


FIG. 144.—Longitudinal section of the intestinal villus of a rat containing cystic stage of *Hymenolepis nana*. (Enlarged.) (*From Stiles and Crane, after Grassi and Rovelli.*)

Stiles and Crane (37) give the following complete description of the species: "Strobila 10 to 60 millimeters in length, 2.5 to 4 millimeters in maximum breadth; composed of 800 to 1300 segments. Head small, almost globular; 200 to 600 μ in width; rostellum rudimentary, pyriform, only slightly protractile; hooks absent; suckers globular, near the apical portion of the head, 80 to 160 μ in diameter. Neck usually short. Segments throughout strobila broader than long. Genital pores on left margin, near the junction of the anterior and middle thirds of each segment. Three testes in each segment; vas deferens dilates into a prominent seminal vesicle before entering the cirrus pouch, within which also is a vesicle. Gravid uterus occupies most of the proglottids; its cavity is subdivided into a large number of incompletely separated compartments filled with eggs. Eggs round or slightly oval; outer membrane 54 to 86 μ in diameter, yellowish in color, may be radially striated; inner membrane 24 by 20 μ to 40 by 35 μ in diameter, with mammilate projection at each pole often not apparent; between outer and inner membranes a prominent third layer of albuminous substance, often appearing as two delicate smooth membranes, with intervening space filled by a granular coagulum; embryonal hooks 11 to 16 μ in length."

The completion of the life cycle requires an intermediate host. This may be one of quite a number of insects, although probably the adult *Tenebrio molitor* and the rat fleas, *Nosopsyllus fasciatus* and *Xenopsylla cheopis*, are the more natural intermediate hosts.

Infection of the primary host occurs by ingesting the infected intermediate host. The cysticercoid which has developed in the intermediate host is liberated in the intestine of its new host and within 18 days it has become attached to the epithelium of the intestine and has developed into an adult worm.

Control measures might profitably be directed toward eliminating any fleas which might be living as parasites on the animals and also toward keeping the feed room or bins free from meal worms, the larvae of the *Tenebrio* beetle.



FIG. 145.—Head and anterior portion of *H. diminuta* from the rat. (Enlarged.) (*From Stiles and Crane, after Zschokke.*)

Hymenolepis microstoma (DUJARDIN, 1845).—This species of mouse and rat tapeworm (Fig. 143C) probably occurs much more rarely than the other two species of *Hymenolepis* that have been described, but it is an interesting form in that the adult is not limited to the intestine. The adults live in the duodenum and the common bile duct which, as described by Joyeux and Kobozieff (24), in intense infestation may be distended until it is as large as the duodenum. The gall bladder also may be completely filled.

Joyeux and Kobozieff (24) have given a complete description of the adult worm. The measurements which they give are: length 80 mm. to 350 mm.; breadth of scolex 200 μ; breadth of rostellum 100 μ; neck 600 μ from base of scolex to first sign of segmentation. There is a simple corona of 27 hooks. Development occurs in several insects, notably in the *Tenebrio* and in the rat flea, *Nosopsyllus fasciatus*.

Dobrovolskaia-Zavadskaia and Kobozieff (10) have described lesions produced by the parasites in the liver and bile ducts. If the ductus choledocus is heavily infected, its wall becomes chronically inflamed and irregularly thickened, and the mucous membrane becomes hyperplastic. However, they state that the hyperplasia of the mucous membrane has never presented a neoplastic character. When the parasite penetrates the liver, it causes destruction of the parenchymatous cells and focal necrosis. In more advanced cases large abscesses develop.

NEMATODES

Many species of nematodes have been reported as parasites of the house mouse. However, a number of these probably seldom would be of impor-

tance in laboratory mice. Only those which occur commonly or have received special attention in the field of experimentation are described here.

Syphacia obvelata (RUDOLPHI, 1802).—Also known as *Oxyuris obvelata.* The caecum of the laboratory mouse is commonly infected with this small oxyurid (Fig. 147) which may also occur in the colon. Upon special examination of the caecae of 34 experimental mice, J. M. Twort and C. C. Twort (39) found 17 infected with this species, while of the colons of 57 of their animals 9 were found to be infected.

Description of the genus, for which *S. obvelata* is the type species, is given by York and Maplestone (46) as follows: "Mouth bounded by three lips; small cervical alae present; vestibule absent; oesophagus club-shaped with a posterior bulb containing a valvular apparatus and separated from the rest by a constriction. Male: with 2 or 3 cuticular "mamelons" on the ventral surface; posterior extremity bent ventrally, body cut away ventrally behind the cloaca and then suddenly narrows and ends in a long pointed tail; narrow caudal alae present limited to the first part of the tail; two pairs of preanal papillae and one pair of postanal pedunculated papillae supporting the alae behind; spicule relatively long and very obvious; gubernaculum directed transversely. Female: tail long and pointed; vulva in the anterior region of the body, behind the excretory pore, and communicating by a short vagina, frequently protruded, with a cuticle-lined ovejector remarkable for the thickness of its muscle coat; uterus single, very long; receptacula seminis parallel and narrow; two ovaries. Oviparous."



FIG. 146.—Cysticercoid of *Hymenolepis diminuta.* (*From Augustine.*)

Measurements given for *S. obvelata* are: male 1.3 mm., female 3.5 to 5.7 mm., eggs 10 μ to 142 μ in length and 30 μ to 40 μ in breadth.

Not much inflammatory reaction is caused by this parasite unless it occurs in large numbers; and if general hygenic conditions are maintained for the laboratory animals, it is doubtful if heavy infection will occur.

Aspicularis tetraptera (NITZSCH, 1821).—Also known as *Oxyuris tetraptera. A. tetraptera* like *S. obvelata* is very commonly found in the intestine of the laboratory mouse, but while *S. obvelata* tends to be more limited to the

caecum, *A. tetraptera* is more commonly found in the colon. Of the mice examined by J. M. Twort and C. C. Twort, 43 of the colons of 57 animals contained this parasite while only 3 of the caecae of 34 mice did. The two parasites may be found together.

These parasites can be distinguished in that the uterus or oviduct of *A. tetraptera* extends posterior to the anus, and also the tail of *A. tetraptera*

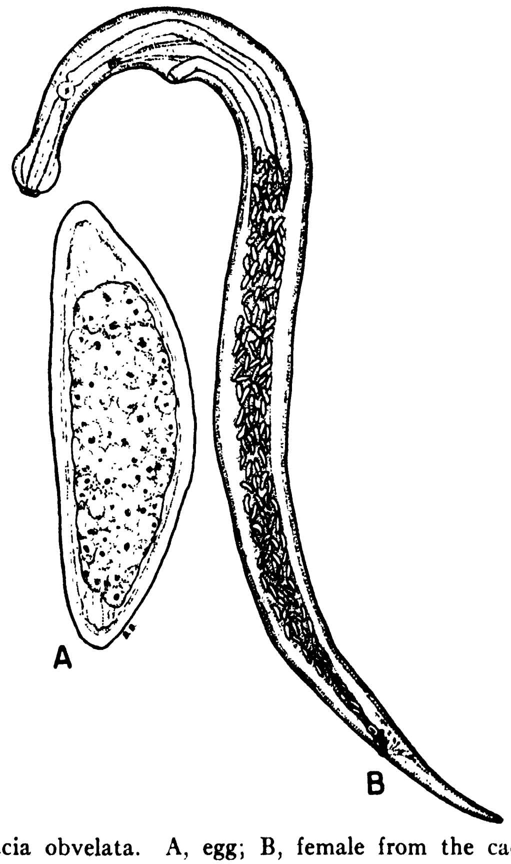


FIG. 147.—Syphacia obvelata. A, egg; B, female from the caecum of a mouse. (Enlarged.) (*From Augustine, after Ripley.*)

is short and bluntly pointed posterior to the reproductive organs, while that of *S. obvelata* is long, extending for some distance posterior to the anus. The male of *A. tetraptera* measures from 2 to 2.5 mm. in length, and the female from 2.58 to 4 mm. The eggs range from 84 μ to 90 μ in length by 34 μ to 40 μ in breadth.

This species is similar to *S. obvelata* in its pathogenesis and control.

Longistriata musculi DIKMANS, 1935.—The trichostrongyle, *L. musculi*, parasitic in the intestine of the mouse, was first described by Dikmans (9) in 1935, and in the same year its life history was reported by Schwartz and Alicata (34). It is a small worm, the adult males measuring 3.25 to 4.5 mm. long and the adult females 4.25 to 6.75 mm. The anterior end of the body is usually coiled in a loose spiral.

In life-history studies Schwartz and Alicata found that the eggs soon after being eliminated from the host hatched into larvae which after but one molt reached the infective stage. Thus, there occurred a deviation from the usual four molts characteristic of the development of nematodes generally. Both infection through the mouth and through the skin resulted in the appearance of worms in the small intestine which developed with two molts into adults. The usual migration through the lungs was not essential for development. In some cases the skin-penetrating larvae did pass through the lungs, but these were considered exceptional.

Nippostrongylus muris (YOKOGAWA, 1920).—Yokogawa (44) first described this parasite from wild Norway rats caught near Baltimore. Of 26 rats taken, 24 were infected. It has also been found in the house mouse caught in the same locality. Although Porter (28) found in a comparative study of *N. muris* in rats and mice that mice were quite susceptible to infection, he concluded that the mouse is a somewhat abnormal host, as demonstrated by the longer prepatent period, smaller percentage development, lower egg production, and shorter duration of infestation in mice than in rats. The parasite has been used extensively for studies of resistance in which the rat has been employed.

The worms appear red, filiform, and somewhat narrowed anteriorly. The adult males are 3 to 4 mm. in length with a maximum thickness of 0.085 to 0.1 mm., and the females are 4 to 6 mm. long with a maximum thickness of 0.09 to 0.12 mm. They are usually found in clumps or nests in the anterior half of the intestine. These nests appear red due to the excess blood in the villi of the region. Yokogawa (45) found that the infective larvae could enter the host both via the mouth or through the skin, the latter being most effective. They go to the lungs where they undergo a part of their development, and later complete their development to maturity in the intestine. The eggs are ellipsoidal with very thin shells. They average $58\ \mu \times 33\ \mu$.

N. muris is decidedly pathogenic if present in large numbers. Africa (1) described heavily infected animals as having been manifestly ill as shown by their emaciated condition. Their eyes were dull and their hair ruffled.

They would shun food placed before them. The stools of the infected animals were soft and mucoid. He found clumps of the adult worms in the intestine usually pinned to the mucosa for considerable depths. Porter (29) found that death of heavily infected animals was due to lobar pneumonia resulting from the migration of the larvae through the lungs similar to that produced by *Ascaris* larvae. He states that in cases of mild infection macroscopically the lungs show small haemorrhagic areas in which the larvae can usually be found, while in severe cases the lungs may be entirely haemorrhagic, congested, and edematous. Microscopically there appear areas of marked consolidation and diffuse haemorrhage.

A compensatory emphysema may be seen in areas in proximity to the migrating larvae. Deposits of pigment were found near the larvae, usually free, but sometimes within the mononuclear leucocytes. He observed that in the intestine the worms migrate extensively in and out among the villi, causing local destruction and shrinking of the tissue, and hinted that the adults may feed upon the glandular secretion or tissues of the host.

A period of at least 5 or 6 days after the egg is passed from the host is required for the infective larva to develop. Thus, it is quite unlikely that heavy infection with this parasite would occur among laboratory animals if the cages are frequently cleaned.

Protospirura muris (GMELIN, 1790).—This species of Spiruridae infests the stomach of mice and rats. It apparently occurs commonly, and often in large numbers. Hall (17) states that he has seen a case in which the empty stomach was distended by a number of these worms which ballooned the stomach walls as so many clock springs might have done. The parasite is quite cosmopolitan in its distribution.

These are rather thick worms with relatively small heads. The males are from 14 to 28 mm. long with a maximum diameter of over 1 mm., and the females are 15 to 40 mm. long with a maximum diameter of 1.75 mm. As with other Filarioidea, an intermediate host is employed in the life cycle, the eggs developing into infective larvae in the body of the meal worm, *Tenebrio*. Thus, control measures should be directed toward the eradication of meal worms.

Gongylonema neoplasticum (FIBIGER AND DITLEVSEN, 1914).—Also known as *Spiroptera neoplastica*. This Spiruridae, which has been reported from Denmark, the Danish West Indies, and the United States, occurs in the squamous-celled anterior portion of the digestive tract of the mouse and rat. Insects including the cockroach and *Tenebrio* serve as intermediate hosts.

The fully developed larvae may be found coiled up in the muscles of the prothorax and limbs. Infection occurs in the primary host by the ingestion of the infected insects.

Fibiger (16) in Denmark has reported extensively on this parasite in relation to its induction of neoplasms in the fundus of the stomach of the mouse and rat and in the tongue of the rat. He describes these neoplasms as possessing exactly the same histological structure as epitheliomata in man and animals.

Trichinella spiralis (OWEN, 1835).—Also known as *Trichina spiralis.* The "trichina worm," well-known as the organism which causes trichinosis, is a parasite of hogs, rats, mice, and other mammals, including man. The adults live in the small intestine and the larvae in the muscle tissue.

The adult is a small worm with the body somewhat tapering anteriorly. The male is from 1.4 to 1.6 mm. long, and the female from 3 to 4 mm. The adult male and female copulate in the intestine of the host, after which the female burrows into the mucosa of the intestine. The female is viviparous, the larvae being deposited in the lymph spaces. The embryos make their way to the voluntary muscle and into the sarcolemma, developing into the infective larvae which assume the spiral form within the lemon-shaped cyst. Muscles with the richest blood supply are said to be most heavily parasitized. Infection occurs by eating muscle tissue containing these infective larvae.

It is highly possible that trichinosis could become a serious disease among laboratory mice if the infestation were permitted to become intense. However, under usual laboratory conditions where few mice are kept in the same cage and where animals are seldom permitted to die in the cages and be eaten by their mates, there is slight chance for intense infection.

Other nematodes of the mouse.—The following are species described for the mouse but of less importance than the above forms.

Capillaria bacillata (EBERTH, 1863).—Reported from the oesophagus of the mouse.

Ollulanus tricuspis LEUCKART, 1865.—Adults live in the gastric mucosa of the cat. Larvae develop in the musculature and connective tissue of the mouse.

Gongylonema musculi (RUDOLPHI, 1819).—Reported from the liver and on the external walls of the stomach.

Heterakis spumosa SCHNEIDER, 1866.—Usually reported from the caecum of the rat, but Harkema (18) found it in the house mouse.

For further description of these species see Hall (17).

Arthropods

Lice

Several different species of lice have been reported as being found on the house mouse. However, the most common louse of the laboratory mouse is probably *Polyplax serrata* (Burmeister) (Fig. 148), recently redescribed by Jancke (23). Specimens taken from the mice at the Jackson Memorial Laboratory have been identified as this species by Dr. H. E. Ewing, U.S. Bureau of Entomology and Plant Quarantine. *P. serrata* is a common parasite of the house mouse in Europe, but has been found only on laboratory mice in this country (14). *Hoplopleura hesperomydis* (Osborn, 1891) has been reported from the house mouse in California, and *H. acanthopus* (Burmeister) occurs on the house mouse in Europe (38, 15). The common species of the rat is *Polyplax spinulosa* (Burmeister) which is cosmopolitan in its distribution (5).

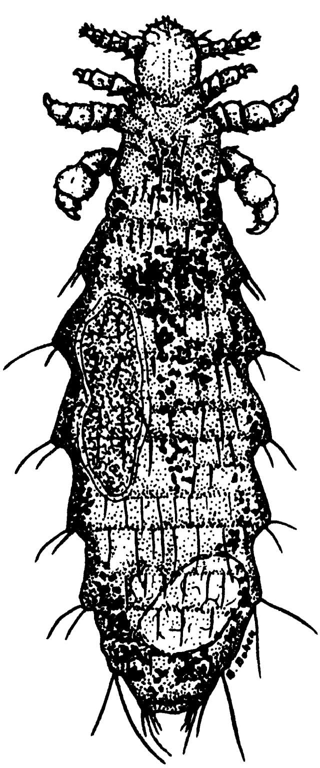


FIG. 148.—*Polyplax serrata*, adult female. Dorsal view (×75).

Lice are permanent ectoparasites. They move slowly and usually pass from one animal to another only when the animals are in contact with each other. Those found on the mouse are of the type with sucking mouthparts, and feed by piercing the skin and sucking the blood of the host. The eggs are elongate and are fastened to the hairs of the host most commonly on the dorsal neck region or on the belly. They can be seen readily by parting the hair, and they afford one of the easiest ways of discovering an infestation. The young are similar to the adults in structure, but are paler in color. After several molts they reach the mature state. Under laboratory conditions with the temperature controlled there is no interruption in their activity, and many generations can be produced each year.

Mice infested with lice usually display a general unhealthy appearance. This is somewhat due to the loss of blood, but probably more to the irritation which the parasites cause making the animals restless and constantly scratching.

Aside from the above detrimental qualities, lice are undesirable because of the danger of spreading disease among the animals. Their method of feeding by sucking the blood of the host facilitates the spread of any organism living in the blood of the host. Eliot (13) has shown that *P. serrata* transmits the blood organism *Eperythrozoon coccoides*. *P. spinulosa* of the rat transmits *Bartonella muris*, and in rabbits, lice act as transmitting agents for tularemia.

Eradicative measures against lice must be applied directly to the mice since the parasites do not commonly leave the host. Insecticides such as sodium fluoride and pyrethrum, both of which are included in many commercial products, are effective. These may be applied by dusting the dry product into the coat of the mouse, or by spraying or dipping the animal into a solution of the insecticide. Spraying is more advisable than dipping, since mice often become chilled following dipping and pneumonia may develop. Small atomizers (perfume atomizers) can be well adapted to spraying mice. Different oils including kerosene are effective, although, especially in the case of kerosene, the amount applied should be limited so as not to irritate the skin of the mouse. Whatever treatment is used should be repeated one or two weeks after the first treatment in order to eradicate any lice which may have hatched after the first application.

Conditions which in general tend toward producing healthy mice are of value in louse control. If a mouse is otherwise in good condition it usually can free itself of any lice. Animals experimentally subjected to adverse conditions, as being fed a deficient diet, are more commonly infested.

FLEAS

Fleas do not tend to be as restricted to a particular host species as do some of the other insect parasites. Thus, it would not be surprising to find any of a number of different species of fleas attacking laboratory mice. However, the species commonly known as the mouse flea is *Leptopsylla musculi* Dugès. This species occurs abundantly on mice and rats in Europe and has been reported from mice and rats in the U.S. (14). The common rat flea, *Nosopsyllus fasciata* Bosc, which is often concerned in plague transmission, is the flea most commonly found on rats in Europe and North America. It also occurs on mice. The Oriental rat flea, *Xenopsylla cheopis* (Rothschild), likewise well known for its role in the transmission of bubonic plague, is cosmopolitan in its distribution, having established itself in several localities in the Midwestern States of the United States.

One can readily recognize fleas as such by their wingless, laterally compressed bodies and their remarkable jumping ability. They feed entirely from the blood of the host, but do not necessarily remain on the host all the time for they are often found in the nest of the host or they may even be found throughout the laboratory. The female lays her eggs in the nesting material or among the hairs of the host. In the latter case the eggs usually have dropped to the bedding of the host before they have hatched. The worm-like larvae are not parasitic but feed on any organic material in the debris in which they live. After remaining in the larval stage for a week or ten days, during which time they molt three times, they pupate within silken cocoons. Under conditions favorable for development such as would be found in a laboratory, the adults emerge from the pupae after another period of a week or ten days.

Fleas irritate their hosts considerably, and danger in flea infestation also lies in the possibility of their spreading disease among laboratory animals. It is well known that fleas transmit bubonic plague and endemic typhus and that they serve as the vector for the rat trypanosome and probably also that of the mouse. Just how many more pathogenic organisms they transmit is not known, but there are probably many.

Regular weekly cleaning of the cages of laboratory mice automatically controls flea infestations as such treatment destroys the developing larvae and pupae. However, the source of flea infestations in buildings can sometimes be traced to a dog or a cat which frequents the building. In such cases the infestation can be controlled by cleaning and disinfecting the bed in which the dog or the cat sleeps.

Bedbugs

In some laboratories the bedbug, *Cimex lectularius* Linné, has adapted itself to feeding on the experimental animals and has proved to be a very disagreeable pest. With the ideal conditions presented by the laboratory and with a constant, abundant supply of food, bedbugs grow vigorously and breed rapidly.

They are dark, flat insects with vestigial wings (Fig. 149). They feed entirely on the blood of the host, being active at night and retiring for the day to cracks and crevices in the cages and racks. Each female lays from 75 to 200 eggs which she conceals in the crevices where she hides. The young bedbugs, or nymphs, are similar to the adults but are paler yellow in color. They too feed on the blood of the host. After molting five times the adult with the rudimentary wings appears.

Just how important bedbugs are in transmitting disease is not known, but it would seem that they might act as transmitters for almost any blood infection of the host or even for those diseases spread through the waste products of the body for they readily travel from one cage to another. In laboratory experiments they have been shown to be capable of transmitting relapsing fever, bubonic plague, and tularemia.

It is putting it mildly to say that the eradication of a bedbug infestation from a laboratory is a difficult task. Fumigation by the use of hydrocyanic acid gas or by burning sulfur is effective in homes, but such treatment in the laboratory necessitates putting all the experimental animals in sterilized cages and removing them to another building or room which would obviously be impractical unless the colony of animals were small. About the best attack is to clean each room systematically and thoroughly, removing the cages of animals from the racks, transferring the animals to sterilized cages and returning them to the racks only after the racks have been painted with kerosene with special attention given to the crevices where the bugs may be hiding. All the crevices in the walls of the room and the cracks in the floor should be thoroughly treated with kerosene. The racks should then be placed so as not to come in contact with the walls. It is even advisable to stand the legs of the racks in cans of the oil. A few days following this procedure an application of a solution of 1 ounce corrosive sublimate to 1 pint alcohol and ¼ pint turpentine can profitably be applied. The solution should be painted into the cracks of the racks or about the room and other places where the bugs are likely to come in contact with it in their attempt to make their way to the animals. This solution retains its effectiveness for some time after the application. Corrosive sublimate is extremely poisonous, and great care should be taken in handling it. The solution should not be permitted to come in contact with the skin. It also has a corroding effect on metals, necessitating the handling of the solution in glass or wooden containers.

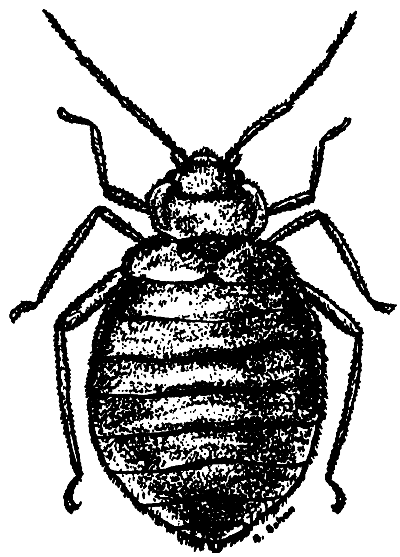


FIG. 149.—Bedbug, *Cimex lectularius* Linne, from the mouse. (×10.)

Laboratories can well be constructed so as to inhibit the establishment of bedbug infestations. Brick walls, concrete floors, and an absence of wooden partitions all tend to eliminate the abundance of cracks in which the bugs might hide. For this reason, metal racks are more desirable than wooden ones.

Other suggestions for bedbug control are given by Back (4).

MITES

Liponyssus bacoti (HIRST).—The mite found most commonly infesting the experimental mice in the Jackson Memorial Laboratory is *Liponyssus*

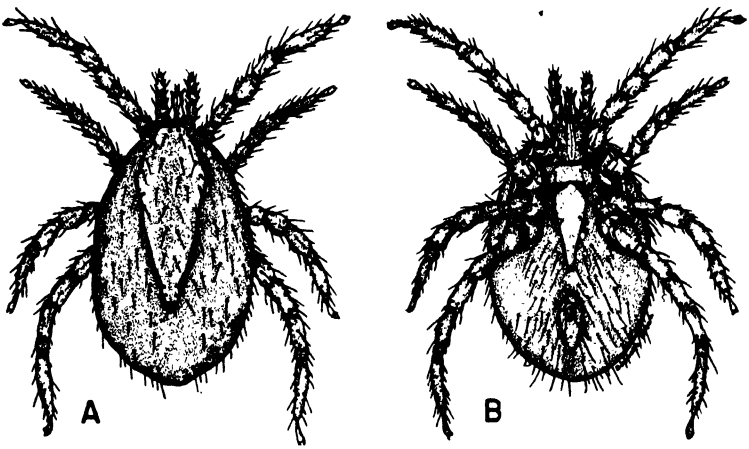


FIG. 150.—Tropical rat mite, *Liponyssus bacoti* (×50). A, dorsal view of female; B, ventral view of female. (*From Dove and Shilmire.*)

*bacoti** (Fig. 150). This is the tropical rat mite, which was first described from Egypt, but which has since been found to occur in many widely separated countries of the world. It has been found infesting rats quite commonly in some of the Southern States where it even frequently attacks man.

Dr. F. C. Bishopp of the U.S. Bureau of Entomology and Plant Quarantine believes that this is the first time that this species has been reported as a pest of laboratory animals. This is especially interesting in view of the fact that the mice from which the present strains at the Jackson Laboratory originated have been known to have been infested with mites since before 1919, and although this is the first time that a specific determination has been made, it seems quite possible that the same species has prevailed. The

* Identified by Dr. H. E. Ewing of the U.S. Department of Entomology and Plant Quarantine.

question arises as to whether or not the species is a rather common pest of laboratory mice throughout the country having thus far escaped the taxonomist's attention.

The mites occur on the hosts for the most part only while they are feeding, and after they have become engorged with blood they retreat to crevices in the cages or racks where they breed. They migrate freely from cage to cage and sometimes even from one room to another. Heavily infested animals develop a scabby skin and rough coat accompanied by a generally poor health condition.

Although it has not been shown that *L. bacoti* transmits diseases of laboratory mice, certainly such possibility exists. Dove and Shelmire (11) have reported that they were able to transmit endemic typhus from guinea-pig to guinea-pig through bites of this parasite.

In the laboratory, unless control measures are applied to these pests, their numbers will reach epidemic proportions. They can be attacked most effectively by thorough cleaning, and by disinfecting the cages regularly. It is well also to spray or paint kerosene into the crevices of the racks. Dusting the animals with pyrethrum powder or spraying them lightly with pyrethrum extract aids in the eradication of the parasite. Using metal cages and metal racks which do not afford good breeding places is a worth-while preventive measure.

Echinolaelaps echidninus (BERLESE).—This is the common rat mite which is found on rats in various parts of the world and especially in warmer countries. It has been reported from the house mouse in the United States. This species has been found to be the vector of the pathogenic haemogregarine (*Hepatozoon muris*) of the rat.

Myobia musculi (SCHRANK).—This mite (Fig. 151) has also been found infesting the experimental mice at the Jackson Memorial Laboratory. (Identification by Dr. H. E. Ewing.) These are small mites less than ½ mm. in length. They can be found clinging tightly to the bases of the hairs of the host by the specially adapted front legs which are enlarged and shortened with a terminal hook for grasping the hair. A pair of long stout bristles extends from the posterior end of the body. They evidently cause considerable irritation to the host especially around the face regions, for infested mice will scratch those regions until the whole area is raw.

These parasites are so small and they cling so closely to the host that it is difficult to discover an infestation before the mouse has mutilated itself until it must be discarded. However, pyrethrum extract sprayed on the other members of the same cage has been found to be effective.

Myocoptes musculinus Koch.—This Acarina has been reported as frequently found on mice, each mite tightly clutching a single hair at its base. In this species it is the last two pair of legs that are modified for hair clasping.

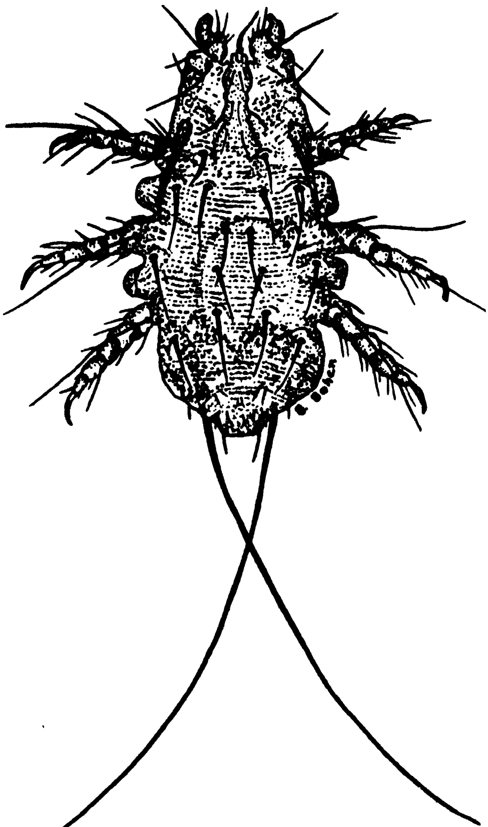


Fig. 151.—*Myobia musculi*, adult, dorsal view.

Ewing (14) notes that after the infested mice are dead these mites will crawl to the tips of the hairs where they are observed as tiny white specks.

Control Measures for Other Insect Pests of the Laboratory

Cockroaches.—One of the most effective ways of eradicating cockroaches is by the use of sodium fluoride. The dry powder should be dusted into cracks of the partitions, behind baseboards, around sinks, under drainboards, and around the pipes and other such places frequented by the insects. The roaches get the powder on their appendages and when cleaning them get it into their mouths, thus becoming poisoned. This treatment is

slow and should be continued for some time or until all roaches are eliminated. Care should be taken not to get the poison into the animal cages.

Silverfish.—These pests can be eliminated by dusting pyrethrum powder or sodium fluoride about the places where they occur. Fresh pyrethrum powder is the more effective in this case.

Meal worms and other grain pests.—These can readily eradicated by fumigating the grain bin or room with carbon bisulfide. One pound of carbon bisulfide to each 100 cu. ft. of space is recommended. It is well to pour the liquid on a cloth and place it in a container in the bin or room. The heavy fumes sink and penetrate the grain thoroughly. The sides and bottoms of the rooms or bins should be as nearly air tight as possible, and it is well to cover the bin, leaving it closed for from 36 to 60 hours. The temperature should be between 75 and 90°F. for best results.

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Chapter 12
INFECTIOUS DISEASES OF MICE

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INTRODUCTION

Mice have been employed experimentally since the early days of bacteriology. Until fairly recently, however, there has been a lack of extensive information regarding their anatomy and physiology, and considerable confusion with respect to the nature, etiological agents, and pathology of their infectious diseases.

It is essential for any investigator who utilizes mice to become familiar with the well animal. Anatomical descriptions are available in this book and elsewhere (133, 110), as well as reports concerning operative techniques (133), induction of narcosis (133), the blood picture (201, 247), temperature (247, 130), intestinal flora (307), and death rate (299) of normal mice, and the effect of starvation on temperature, blood, etc. (130).

Equally imperative is it that he be acquainted with the clinical signs and pathological features of their spontaneous diseases. Such knowledge may avoid embarrassing confusion by the recognition of a latent infection whose manifestations might otherwise be misleading in the evaluation of experimental data or in an attempt to isolate an etiological agent from other hosts. Moreover, with early recognition of disease in valuable stock, such as genet-

ically pure strains, steps may be taken to prevent spread of the infection and destruction of the colony. The mouse must also be regarded as a reservoir of certain animal and human diseases. These considerations have led to the inclusion in this volume of a summary of the literature concerned with the natural diseases to which mice are subject. Other general descriptions of diseases in mice may be found in the works of Jaffé (110), Meyer (172), and others (299, 87, 170).

Bacterial Diseases

Mouse Typhoid

In 1890, a highly fatal epidemic occurred in the laboratory mice of the Hygienic Institute at Greifswald, 69 per cent of the animals succumbing (154) to the infection. Loeffler noted the large, brownish-red spleens and the small yellow lesions in the livers of dead animals. Groups of organisms in capillaries reminded him of typhoid bacilli in human tissues. His studies of this bacillus showed that it was closely related to the colon-typhoid group and accordingly the name "*B. typhi murium*" was proposed. Subsequent investigations have revealed that this strain and related organisms of the paratyphoid (*Salmonella*) group produce one of the most important bacterial diseases of mice.

Occurrence.—Mouse typhoid occurs so commonly in rodents that only with elaborate precautions can a colony be maintained free from infection. Animals suffering from the chronic form of disease may harbor and excrete the organism for months, thus maintaining infection in the colony; or the infection may be introduced from without in the food or from wild rodents gaining access to the animal rooms. *Salmonella typhimurium* and *S. enteritidis* are found with about equal frequency in apparently healthy wild and laboratory mice and as the cause of epidemics (47, 235, 335, 236, 113, 10, 159, 336, 26). The incidence of carrier infection in stock mice may vary from 1 to 20 per cent and rarely to 100 per cent (307, 172, 330). Other paratyphoid strains are uncommon, although epidemics due to Morgan's bacillus (*Proteus morgani*) (337) and an unknown species of *Salmonella* (265) have been reported.

The natural disease.—*Salmonella* infections in general are bacteremic diseases and may run acute, subacute, or chronic courses. The various bacilli produce essentially the same clinical picture. Infection as a rule takes place by the oral route and the incubation period extends from 3 to 6 or more days. The first sign of illness is a loss of normal activity and

industry—the animal sits quietly in a corner of the cage, frequently hunched over with his head bent down. His hair becomes ruffled and loses its normal gloss; anorexia develops to a varying degree though usually not complete, and loss of weight occurs. Later, a conjunctivitis may develop, the eyelids become glued together, and the respiratory rate is accelerated. The feces are usually formed, but are softer and lighter in color. A few animals become hyperexcitable shortly before death. The temperature usually

Table 1

THE EFFECT OF INFECTION WITH *S. typhimurium* ON THE LEUKOCYTES OF THE MOUSE

	Total Leukocytes	Differential (%)				
		Lymphocytes	Monocytes	Neutrophiles	Eosinophiles	Immature Granulocytes*
Normal Mice (Average)	7500	66.5	5.5	26.4	1.4	6
Infected Mice						
Mouse No. 1						
1st day	7200	78.4	5.2	15.0	1.4	6.6
7th day	5200	17.1	5.7	77.2	...	21.7
Mouse No. 2						
1st day	7000	73.5	4.5	20.9	1.1	6.6
7th day	5400	24.3	5.4	70.3	...	18.1

* This figure represents the percentage of granulocytes which are immature. Taken from Seiffert, Jahncke, and Arnold (247).

remains within normal limits, although a terminal rise and fall may occur (247). The blood picture shows a leukopenia with an increase in granulocytes, many of which are young forms, and a decrease in lymphocytes (Table 1). Increased polychromatophilia and slight poikilocytosis of the erythrocytes are present.

In the acute form the disease progresses rapidly and death may occur within a week, the above signs occurring almost simultaneously or in rapid succession. In the chronic form, there may be no signs of illness, or only those of a mild infection followed by apparent recovery, or of a slowly progressive cachexia. The presence of infection can be verified, however, by isolation of the specific organism from feces and organs of the animals months

later (280, 281, 283). All degrees between these extremes are met with, depending upon such variables as the dosage, resistance of the individual mouse or strain of mice, virulence of the organism, and external environmental factors such as temperature (129), and diet (329, 172).

The experimental disease.—In part because of its similarity to human typhoid fever, the pathogenesis of *Salmonella* infection in mice has been extensively studied experimentally (247, 336, 177, 139, 300, 190, 192, 191, 255). After oral administration, a transitory excretion of the bacilli occurs in the feces. The organisms do not multiply and many are excreted from or destroyed in the gastro-intestinal tract, since a period follows in which no organisms can be recovered from the stools. Invasion of the lymphatic system then occurs, with involvement of the intestinal lymph follicles, mesenteric lymph nodes, and less often the tracheobronchial and cervical nodes. Here multiplication presumably occurs, bacteria are carried by lymphatic channels such as the thoracic duct to the blood stream, and a transitory bacteremia ensues (second to fourth day), terminated by removal of the bacilli through action of the reticulo-endothelial cells, particularly in the liver and spleen. Bacterial proliferation occurs in the lymph nodes, liver, and spleen for the next 2 to 4 days, as evidenced by the increase in numbers of bacilli found in these organs, but the blood remains sterile. Finally, a progressive re-invasion of the blood stream occurs with generalization of the infection throughout the body and secondary invasion of the intestine. Bacilli are found in such tissues as muscle, gall bladder, bile, and urine after the septicemia has become established. Direct invasion of the blood stream occurs only when the infecting strains are of highest virulence and toxicity or when an overwhelming dose is given. Following the second bacteremia, the bacilli multiply rapidly in the intestine and may overwhelm the normal flora (307). In cases of chronic infection, such as is present in mice which have survived natural or experimental infection, organisms persist in the spleen, liver, lymph nodes, and gall bladder for months, and are intermittently or continuously discharged in the feces (see work of Amoss, Neufeld, Topley, and Webster). The various strains of *Salmonella* behave similarly (177, 5, 6). Fatal infections may also be produced by applying bacilli to the depilated intact or lightly scarified skin, to the mucous membranes of the conjunctiva by dropping a liquid suspension into the eye, or by inhalation.

The role played by "toxins," lytic products, or specific substances derived from the bacteria is difficult to define, but presumably such products of the organism account for the leukopenia and focal necrosis in the liver.

Cameron, Delafield, and Wilson (43) have recently demonstrated that a toxic substance obtained from *S. typhimurium* by tryptic digestion produces congestion, disappearance of glycogen, and focal necrosis of the liver, and early necrosis of Malpighian bodies and of lymphoid follicles in the lymph glands.

The mortality in spontaneous and in experimental epidemics varies from 20 to 80 per cent, and is influenced by the strain of organism, dosage or multiple exposures, resistance of the stock, age of animals, season, and other factors (159, 308, 316, 329, 206, 207, 211, 277, 279, 181).

Pathology.—The pathology of this infection has been studied in mice dying of the spontaneous disease, but, more satisfactorily, in mice experimentally infected by mouth under controlled conditions (154, 177, 190, 110, 247, 87, 26, 196). In this way it has been possible to compare the findings in acute and chronic infections, and to follow the course of chronic disease by daily examinations (247).

In very acute infections such as result from massive doses, the pathological findings are not characteristic but resemble those of any septicemic disease. Gross examination reveals congestion of all blood vessels and viscera, some enlargement of liver and spleen which are usually dark red in color, occasionally serosanguineous fluid in the peritoneal cavity, slight to moderate enlargement of the lymph nodes, and redness, injection, and swelling of the intestinal mucous membrane. Microscopically, the findings are those of hyperemia and congestion of all the organs, fatty degeneration in the liver, and severe catarrhal inflammation of the intestinal mucous membrane. Bacteria may be found in large numbers in the blood, peritoneal exudate, and the various tissues. Focal lesions are infrequently found in animals dying before the fifth day.

Animals living 1 or 2 weeks or longer show more typical lesions. Emaciation is usually pronounced and the abdomen appears enlarged due to increase in size of the liver and spleen and to intestinal distention. On opening the body, the vascular congestion is seen to be less pronounced than in the acute infection. The liver is enlarged and the spleen may extend down to the level of the pelvic bones. The peritoneal and thoracic cavities may be free from fluid or contain small amounts of bacilliferous serous, serofibrinous or sanguineous (154) exudate. The intestinal serosa is usually reddened and injected, and the content of the bowel varies from thin, watery, yellowish material containing mucus to soft or normal scybalae.

The pathology of the gastro-intestinal tract can be correlated quite well with the stages in the pathogenesis of the infection given above. During

the first 2 to 4 days, slow enlargement of the solitary lymphoid follicles, Peyer's patches, and mesenteric lymph nodes occurs, with catarrhal inflammation of the mucosa appearing during the latter part of this period. After blood stream invasion and the appearance of symptoms, the mucosa becomes progressively red and swollen, mucus appears in increasing amounts and hemorrhages into the mucosa and lumen are found. Ulceration of lymph follicles appears. Similar but less marked changes are found in the stomach, especially in the pyloric portion. Microscopically, the picture is that of a severe enteritis—capillary injection, denuding of epithelium, ulceration of lymphoid follicles, and infiltration with polymorphonuclear leukocytes and histiocytes. The mesenteric lymph nodes are swollen, congested, hemorrhagic, and often show focal necrosis. Cellular infiltration and bacteria may be found from the first few days of the infection.

The spleen is regularly enlarged to three or four times its normal size. In color, it is dark red or reddish-purple; its capsule is tight and its consistency firm. Rarely, yellowish-white nodules may be seen under the capsule. On sectioning, the pulp protrudes and the cut surface is mottled with irregular hemorrhagic and gray areas. Histologically, congestive hyperemia, increase in pulp cells, degeneration of lymphoid cells in Malpighian corpuscles, infiltration with inflammatory cells, and occasionally focal areas of necrosis are the chief findings. Intra- and extracellular bacteria are present after the fourth day.

The liver enlarges progressively up to twice its normal size during the course of the disease. It varies from a deep red to a brownish-yellow color and is friable in consistency. The capsule is usually smooth but, when peritonitis is present, it may be covered with a fibrinous exudate containing bacteria. From the fifth or sixth day on, small, yellow, pin-head sized lesions appear and increase in size and number. Microscopically these consist of foci of lymphoid-like cells which begin to form about the second day. The foci increase in size and then become necrotic. Liver cells surrounding them also become necrotic, but retain their normal structural alignment. The areas remain fairly well circumscribed, but are infiltrated at the periphery with granulocytes and histiocytes. Occasionally, fibrinous thrombi are found in the hepatic capillaries, and capillary hemorrhage into the parenchyma is irregularly present. Bacteria are found extracellularly in the tissues, intracellulary in neutrophiles and histiocytes, and frequently in clumps in the capillaries.

The lungs may be entirely free from involvement, may show punctate hemorrhages, or may be congested and hyperemic. Histologically, the

capillaries are not infrequently distended with mononuclear cells. Pneumonia has been produced experimentally by aspiration of *S. enteritidis* (335).

The bone marrow shows considerable damage, apparently due to the depressant action of a toxic substance liberated from the bacilli. Maturation of the granulocytes is disturbed, and in severe cases a practically complete granulocytic aplasia may be present. It is possible that these organisms elaborate a leukopenia-producing substance such as has been isolated from the typhoid bacillus by Morgan (174). Complex polysaccharide-phospholipids which are highly toxic and produce hyperglycemia have been isolated from *Salmonella* strains, but their effect on bone marrow and leukocytes has not been reported. (See Topley and Wilson (287), pp. 566–568 for discussion.)

Pathological changes in the remaining organs are inconstant and are mainly the result of congestion or hyperemia. Occasionally, bacillary emboli can be found in the glomerular tufts of the kidney and elsewhere in capillaries. Focal inflammatory areas may be present in the myocardium. Bacilli are almost always seen in considerable numbers in smears of the blood, liver, spleen, and lymph nodes.

Etiology.—As already pointed out, the organisms most commonly found in cases of mouse typhoid are *Salmonella typhimurium* and *Salmonella enteritidis*. Other strains of the paratyphoid group occasionally cause sporadic deaths in laboratory or wild mice, but epidemics due to them are rare.

In general, the organisms of this group are similar in morphology and biochemical reactions which accounts for the several names given to various strains and much of the confusion concerning them in the older literature. The early work of Smith (254), Bainbridge (16), TenBroeck (266), and others (134, 243, 282, 284) did much to clarify the situation. More recent studies of antigenic structure have provided a logical basis for the classification which has been accepted by the Salmonella Subcommittee of the Nomenclature Committee of the International Society for Microbiology (232).

The *Salmonella** organisms are gram-negative rods, usually motile, which grow aerobically on ordinary media, and form acid and gas from the carbohydrates which they ferment. They do not attack lactose, sucrose,

* The classification and nomenclature here used are taken from Bergey's "Manual of Determinative Bacteriology" (22), and are based on those of the Salmonella Subcommittee (232).

or salicin, nor do they ordinarily form indol or liquefy gelatin. *S. typhimurium* is considered to be identical with *B. typhi murium*, *B. aertrycke*, *B. pestis-caviae*, *B. paratyphosus B*, Mutton type, and *B. enteritidis* Breslau of the German literature. Of the several varieties of *S. enteritidis*, only two are important as mouse pathogens: *S. enteritidis* Gaertner (*B. enteritidis*

Table 2

COMPARISON OF THE CHIEF DIFFERENTIAL BIOCHEMICAL REACTIONS AND ANTIGENIC COMPONENTS OF ORGANISMS CAUSING MOUSE TYPHOID OR ENTERITIS*

Organism	Biochemical Reactions											Antigenic Components		
	Maltose	Lactose	Sucrose	Inositol	Glycerol	Litmus Milk	Indol Formation	Nitrate Reduction	H_2S Production	Stern's Medium	*d* Tartrate	O Antigen	H Antigen: Specific Phase	H Antigen: Nonspecific Phase
S. typhimurium	⊕	o	o	⊕	+	Alk.	D	o	⊕	±	±	IV, V, XII	i	1, 2, 3
S. enteritidis														
Var. Gaertner	⊕	o	o	o	⊕	Alk.	o	⊕	⊕	⊕	⊕	IX, XII	gom	
Var. Danysz	⊕	o	o	o	⊕	Alk.	o	⊕	⊕	o	⊕			
Salmonella sp. Syverton and Olitsky (265)	⊕	o	o	o	?	Acid	⊕	⊕	o	?	?			
Proteus morgani (Morgan's Bacillus No. 1)	o	o	o**	o	?	N or Alk.	⊕	?	⊕	?	?			
Esch. coli.	⊕	⊕	±	o	±	Acid	⊕	⊕	⊕	?	?			

⊕ = Acid and gas or positive reaction.
± = Variable or delayed reaction.
o = Negative reaction.
? = Not recorded.
D = Doubtful, probably negative.
\+ = Acid, no gas.
** = Occasional strains show delayed fermentation.
Alk. = Alkaline.
N = Neutral.
* For more detailed information and literature, see Bergey (22) and Topley and Wilson (287).

Gaertner) and *S. enteritidis* var. Danysz (47). The two types are identical serologically and differ only in that the Gaertner variety ferments glycerol in Stern's medium. Although frequently confused in the literature, both types are pathogenic for rodents. In Table 2, the chief differential biochemical reactions of the organisms discussed in this section are tabulated, together with the antigenic structure of the *Salmonella* types.

The simplest method for tentative diagnosis is to culture the heart's blood, spleen, liver, or intestinal contents on one of the selective media which differentiate the non-lactose-fermenting from the lactose-fermenting colonies (Endo or eosin-methylene blue agar plates) or which inhibit the coliform organisms (McConkey, bismuth sulphite, or desoxycholate agar plates). Single colonies of gram-negative bacilli may then be fished to Russell's double sugar slants and subsequently to lead acetate medium. The production of acid and gas in the stab or butt portion of the former and of black lead sulphide in the latter tentatively identifies the organism as one of the *Salmonella* group. Further identification may be accomplished by the biochemical reactions (as outlined above) and the determination of the antigenic structure either by cross-absorption tests with known agglutinating antisera or by agglutinations with antisera previously absorbed to remove all but specific antibodies.

Infections with related organisms.—Four unusual epidemics in mice due to *Salmonella* or related organisms have been reported. Sangiorgi (233) noted a spontaneous disease which involved approximately 20 per cent of his white mice and was apparently due to a coliform bacillus. The affected animals showed ruffled hair, shallow respirations, subnormal temperature, weakness or paralysis of the hind extremities, marked emaciation, and profuse diarrhea with staining of the perianal region. The pathological findings were not unlike those described above: grayish-white necrotic lesions in the liver, hypertrophied and congested spleen, pale kidneys, intestines filled with yellowish, foamy, liquid contents, and serous exudate in the abdominal cavity. The organisms cultured from the heart's blood and spleen were short, gram-negative rods which produced acid and gas in glucose, maltose, lactose, and saccharose and formed indol. Inulin and starch were not fermented. Colonies on Drigalski medium showed the typical red color of colon bacilli. These characteristics placed the organism in the colon group. White mice fed cultures of the bacillus died in 5 to 6 days with a profuse diarrhea. The pathological findings were identical with those of the spontaneous disease.

Spontaneous infections with Morgan's bacillus have occurred in laboratory mice fed on a diet of oats and raw beef (337). The mice displayed an appearance of "unthriftiness and lethargy" which was unlike that of mice in other epidemics. The chief signs were a rough and shaggy coat, hunched up posture, retracted abdomen, anorexia, and occasionally antemortem convulsions. At autopsy, there was a general pallor and dryness of the tissues, the heart was dilated and flabby, the liver nutmeg, and the kidneys

pale and swollen. The disease resembled a chronic intestinal infection in which the etiological agent was unable to penetrate the intestinal mucosa and invade the body tissues. The mortality among infected animals was 100 per cent. A motile, gram-negative bacillus isolated at autopsy was identified as Morgan's bacillus. This organism closely resembles those of the *Salmonella* group in many of its properties, although it is now classified in the proteus group (*Proteus morgani*) (287, 22). Attempts to feed mice and reproduce the infection experimentally almost completely failed, so that a part, at least, of the etiological factors remains unknown.

Syverton and Olitsky (265) have carefully studied an unusual epidemic of acute intestinal infection sharply limited to suckling and newly weaned Swiss mice. The clinical signs were those of profuse diarrhea with apparent tenesmus and marked inanition, rapid loss of weight and tone, complete prostration in 24 to 72 hours, and death in about one-half of the affected animals. Obstipation and cessation of suckling sometimes supervened to produce a state of dehydration that was almost uniformly fatal. If recovery occurred, necrosis and sloughing of the perianal tissues frequently followed. In the absence of obstipation, recovery was usually complete. The age incidence was from 7 to 24 days.

The gross pathological changes varied from slight hyperemia to necrosis of the lower ileum and colon. The spleen was dark red and contracted; in recovered cases it frequently showed gross cicatrization. Microscopically, characteristic changes were present in the lower ileum and colon. The involvement of the intestines was extensive and consisted chiefly of leukocytic and erythrocytic infiltration and generalized ulceration of the mucosa to such an extent that the latter was often found as a slough in the lumen of the bowel. Hypertrophy of Peyer's patches was particularly marked. The spleen was hemorrhagic with varying degrees of necrosis of the pulp cells. Diffuse parenchymatous and fatty degeneration and localized hemorrhages were noted in the liver. Renal congestion, localized hemorrhages, and degeneration of tubular epithelium were present. In the brain, minute focal hemorrhages were frequently found.

From the intestine, and less frequently from the heart's blood, liver, and spleen, organisms of the genus *Salmonella* were cultured. Of 20 strains, 13 were serologically identical; the other 7 behaved serologically as a single variant. All were culturally identical. Significant cross-agglutinations were not obtained with antisera against known *Salmonella* strains. Specific agglutinins for both types were present in the sera of one-third of the recovered mice, but not in those of normal animals. The disease

could be reproduced experimentally in young mice, but not in adult animals unless massive doses were employed. Infection by contact was possible. Virus or parasitic agents were not demonstrable. Fecal carriers appeared probable, since the organism was found in the intestinal contents of the recovered mice.

The organism differed from known *Salmonella* species in the formation of indol, the fermentation of carbohydrates, and its specific serological reactions, and was tentatively classified in the Asiaticus group of the genus (44).

Antoine and Regnier (10) have reported an epidemic of a septicemic *Salmonella* infection which was unusual because of the presence of ocular lesions. Following a conjunctivitis, the ocular and periocular tissues became involved, producing a characteristic facies (ram's face—"Museau de bélier"). Hemorrhagic visceral lesions were present and both types of lesions were reproduced experimentally. The organism was not further identified.

Prevention.—Attempts to evaluate immunization against this disease have chiefly been carried out in conjunction with studies of experimental epidemics in mice (Neufeld, Topley, Webster, and their coworkers). In general, it may be said that the use of killed vaccines (286, 315, 289) or bacteriophage (290, 288, 186) is not effective in preventing fatal infection or spread of the disease when vaccinated and normal mice are in close contact. In many cases the chief evidence of increased resistance is merely a prolongation of life (182, 140, 107). The vaccination itself may be fatal (336). Furthermore, the presence of agglutinating antibodies in sera of recovered or vaccinated mice is not necessarily correlated with resistance (308, 289). Surviving animals may, however, be more resistant to a subsequent inoculation by virtue of specific (289) or nonspecific (308) protective factors.

Vaccination may be of some value in preventing epidemic spread in a valuable animal stock which is already infected (159, 205, 105). In the Rockefeller Institute's cancer stock, the survivors of two epidemics of mouse typhoid due to *S. enteritidis* and *S. typhimurium* (*B. aertrycke*) were vaccinated with a killed suspension of both organisms. No further outbreaks occurred, although a high fecal carrier rate persisted for *S. enteritidis* and a low rate for *S. typhimurium*. It is possible that vaccination contributed, in part at least, to the disappearance of epidemic outbreaks. A similar contaminated stock would be wholly unsatisfactory, however, for such experimental studies as protection tests and virulence determinations,

since the introduction of test material might be sufficient to light up the latent *Salmonella* infection, with erroneous and misleading results.

There are no satisfactory methods at the present time for the eradication of the disease from an infected colony. In some cases the entire herd must be destroyed and a new stock obtained; in others, a *Salmonella*-free stock may be obtained by quarantining the entire colony, dividing it into small units of 5 or 6 mice, destroying all the mice in any unit in which a death occurs from mouse typhoid, and finally destroying units in which fecal carriers are present as determined by repeated cultures. The second procedure is expensive and in many cases impracticable because of the labor involved. Once a stock is obtained free from infection, it can be maintained by adherence to general preventive measures which will be outlined later in the chapter.

Septicemic Diseases of Mice: Pasteurellosis, Pseudotuberculosis, Mouse Septicemia

Mice are highly susceptible to at least three types of septicemic diseases, namely, pasteurellosis, pseudotuberculosis (*Corynebacterium*) and "mouse septicemia" (*Erysipelothrix*). Spontaneous epidemics, however, are uncommon, although sporadic deaths are not infrequently encountered. In general, the diseases run an acute course and thus may not produce characteristic morbid changes, so that the diagnosis must usually be made by isolation and identification of the etiological agent. The three types will be discussed separately, in conjunction with diseases related by virtue of their character or the nature of the infecting agent.

Pasteurellosis.—Diseases due to *Pasteurella* organisms are primarily endemic in wild animals and include the so-called "hemorrhagic septicemia" group, pseudotuberculosis of rodents, and plague. All three types may occur in mice, but only the first is of much importance.

Hemorrhagic septicemia in mice.—Of all the septicemic diseases of animals, one type may be differentiated, since it is characterized by septicemia, capillary hemorrhage, serous, fibrinous, or sanguineous exudation, and the presence of short bipolar-staining organisms. The disease is found in a wide variety of animal species and occurs in spontaneous epidemics in mice (285, 278, 94, 93, 19).

Detailed clinical and pathological descriptions of the spontaneous disease in mice are lacking. The illness usually is acute, death occurring a few hours after the onset of signs which are nonspecific—apathy, ruffled coat, anorexia, conjunctivitis, rapid respiration, etc. The disease is contagious,

spreads readily to normal animals by contact, presumably by means of respiratory and conjunctival secretions, and is dependent on carriers for its continuation. The mortality varies between 75 and 100 per cent. Postmortem findings consist of subserosal and submucous hemorrhages, fibrinopurulent exudations in the pleural, pericardial, and peritoneal cavities, and hemorrhagic consolidation of variable extent in the lungs. The spleen is seldom enlarged and other visceral organs show little or no gross change beyond the exudate over their surfaces. In the more chronic disease, enlargement of the lymph nodes and small necrotic foci in the liver may be present. *Pasteurella* organisms are found in large numbers in the blood, spleen, and inflammatory exudates in acute cases, but may be obtained only with difficulty from animals surviving for several days or longer.

Experimentally, mice are readily infected by *Pasteurella* of this group regardless of the animal source of the organism. Numerous routes of administration are effective—conjunctival, dermal, subcutaneous, intraperitoneal, intravenous, oral, and respiratory (139, 300, 173). Parenteral administration produces an acute, fulminating septicemia terminating by death in 1 or 2 days. Postmortem findings consist of local edema and congestion, fibrinous exudate over the serous surfaces, enlargement of the spleen, and pulmonary edema and congestion. Administration by other routes results in a more chronic infection, lasting up to a week or longer and characterized by more pronounced local reactions depending somewhat on the route of administration. Pathological changes are similar to those found in the spontaneous disease.

The causative agent is *Pasteurella muricida* (*B. murisepticus*) (22, 173). Morphologically, the organism is a short, oval, bipolar gram-negative rod, which is non-motile and measures about 0.3 μ in width and 1.25 μ in length. Growth occurs aerobically on ordinary media at a wide range of temperatures (20° to 37°C.). No growth occurs on bile media. Dextrose, levulose, galactose, sucrose, and mannose are fermented with the production of acid; indol is formed and nitrates reduced. Serologically, this organism cannot be distinguished satisfactorily from other members of the hemorrhagic septicemia group isolated from different animal species and named accordingly. Topley and Wilson (287), however, have found two distinct types of *P. muricida*, distinguishable by agglutination and maltose fermentation.

Diagnosis of the disease in the acute form can usually be made only by identifying the organism, since the clinical and pathological findings are not specific. In chronic cases, recovery of the organism by culture often fails and inoculation of a normal mouse or guinea pig with tissue emulsions

(spleen, lung, blood, mediastinal lymph nodes, etc.) may be necessary. Differentiation of the organism from *P. pseudotuberculosis* and *P. pestis* is based on the characteristics given in Table 3, although considerable individual variation in reactions occurs.

A similar disease occurring as a spontaneous epidemic among mice in the outskirts of Astracan has been reported (67). The causative organism resembled the *Pasteurella* morphologically, but produced acid and gas in glucose, acid in lactose, mannitol, and dextrin, and failed to form indol. It was highly pathogenic only for mice.

Table 3
DIFFERENTIAL CHARACTERISTICS OF *Pasteurella muricida*, *P. pseudotuberculosis*, AND *P. pestis*

Organism	Production of Acid from							Litmus Milk	Indol Formation	Growth in Bile Salt Medium	Motility at 20°C.	Pathogenicity for White Rat
	Dextrose	Sucrose	Sorbitol	Mannitol	Maltose	Glycerol	Rhamnose					
P. muricida	+	+	+	± *	± **	o	o	Neutral	+	o	o	+
P. pseudotuberculosis	+	± **	o	+	+	+	+	Alkaline	o	+	+	o
P. pestis	+	o	o	+	+	± *	±	Neutral	o	+	o	+

\+ = Positive.
o = Negative.
± = Variable.
* Usually positive.
** Usually negative.

Control of the disease is accomplished chiefly by general preventive methods. Animals vary in their individual resistance to the disease and survivors of epidemics are relatively immune (93). Although some immunity can be produced by vaccination with heat- or chemically-killed organisms, it is doubtful whether such a measure would be effective in eliminating the disease from a stock.

Pseudotuberculosis of rodents.—Spontaneous infection due to *Pasteurella pseudotuberculosis* (*B. pseudotuberculosis rodentium*) occurs but rarely and sporadically in the mouse (202, 195), although it is common in other animals (203, 244, 176). This disease is not to be confused with pseudotuberculosis of mice due to *Corynebacterium pseudotuberculosis* (see following section). Mice are susceptible to experimental infection, death occurring within 1 to 3 weeks after inoculation. The course may be rapidly fatal with septicemia, or chronic with signs of enteritis. Natural infection occurs

by the enteral route and the pathological lesions consist of whitish-grey nodules in the intestinal lymph follicles, swelling and caseation of mesenteric nodes, and enlargement of the liver and spleen which contain numerous nodules varying in size. After subcutaneous inoculation, caseation develops locally and in the regional glands. Grossly the lesions may resemble those of tuberculosis or *Salmonella* infections. Microscopically, however, the lesions are exudative in character and consist of central necrotic material and bacilli surrounded by a zone of leukocytes and histiocytes. In the liver foci of degenerated hepatic cells may be found. The organism is distinguished from *P. pestis* with difficulty, both culturally and serologically.

Plague in mice.—Infection with *Pasteurella pestis* is very rare in mice. Sporadic cases and epidemics, however, have been reported among field mice in Mongolia and in the Kirghiz Steppes (339), where the disease is maintained by rodent host-reservoirs. Typical hemorrhages and buboes were found at autopsy, and the pest bacillus was isolated from nodules in the viscera.

Pseudotuberculosis of mice.—Pseudotuberculosis is a term applied to a number of diseases in which the gross lesions resemble those produced by the tubercle bacillus. Its etiology is varied and includes such agents as *Salmonella* organisms, *P. pseudotuberculosis* (see preceding section), parasitic infections, and others. The form described here is limited to mice and is produced by an organism of the genus *Corynebacterium*. It was first reported in 1894 by Kutscher (135), who isolated the bacillus from the lung of a mouse dying spontaneously.

The natural disease.—Sporadic infection is usual in this disease, but mild epidemic spread may take place in laboratory stocks of mice (25, 8, 216, 286, 94). Its occurrence is relatively infrequent; its course chronic in character. Existence of the infection is frequently suspected by the discovery of a caseous lesion of the lung or a lymph node of an otherwise apparently normal animal. Infection presumably occurs by the respiratory or enteric route, carriers and the rodent habit of cannibalism serving to maintain the disease. Distribution of the organism in the animal's body is by way of the blood stream.

Kutscher's original description gives an excellent picture of the usual postmortem findings. The upper lobe of the right lung was transformed into a greyish-white, friable, caseous mass, with marked inflammatory change in the remainder of the lung. Multiple small nodules, resembling tubercles in appearance and consisting of inflammatory foci, were present in the left lung. The only other significant findings were a massive right

pleural effusion and a slightly enlarged spleen. Organisms were abundant in the caseous mass in the right lung and in the nodules of the left lung.

Pulmonary lesions are almost always found in the severe disease, and, in fact, may frequently be the only signs of the disease (287, 25). Involvement of the lung varies from pin-head sized lesions to caseation of an entire lobe associated with pleural effusion. Recent miliary lesions have a transparent greyish-blue center surrounded by a dark red inflammatory zone which is sharply outlined. Microscopically, the normal pulmonic architec-



FIG. 152.—Pseudotuberculosis of mice. Spontaneous infection. Viscera of mouse (*in situ*) showing lesions. (*From Bongert.*)

ture is not visible in the nodules. At the periphery of the lesions numerous bacilli are seen, both intra- and extracellularly. The liver infrequently contains yellowish-white caseous nodules which are raised when located in the subcapsular region, thus differing from the necrotic foci characteristic of *Salmonella* infection. Glandular enlargement and caseation, either focal or generalized, may be found particularly in the mediastinal, mesenteric, and cervical nodes. Isolated nodules may occur in the spleen and kidneys. Occasionally, only the abdominal viscera are involved (Fig. 152).

The experimental disease.—Experimentally, the disease is specific for mice, and can be produced by subcutaneous, intraperitoneal, intrathoracic, and oral routes, and by inhalation. Death usually occurs in 3 to 5 days and rarely later than 14 days, even following infection *per os*. The pathological findings vary somewhat with the route of infection. Generalized infection follows parenteral inoculation, a local abscess occurring after

subcutaneous injection. The lesions are similar to those in the spontaneous disease, except that the lungs are seldom infected. Inoculation into a serous cavity results in a rather characteristic granular exudate over the serosal surfaces, which may take the form of tiny, discrete nodules or coalesce to form a membrane. The liver is infrequently affected, but lesions occur in the spleen and kidneys. Perinephric abscesses and pyonephritis may rarely be present. Diaphragm, heart, voluntary muscles, and subcutaneous tissues may all show nodules. It is interesting, in view of the polymorphism of this organism and its similarity to the streptothrix, that involvement of the joints may occur (216). Infection by mouth produces lesions chiefly in the mesenteric glands and occasionally in the abdominal viscera.

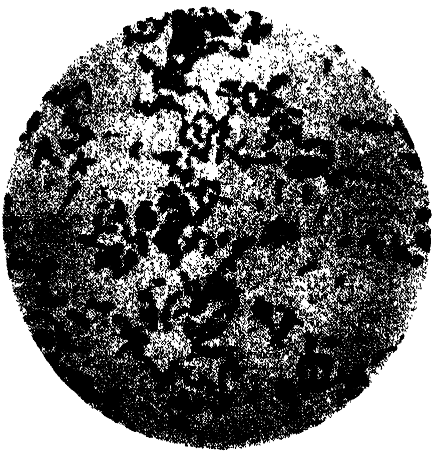


FIG. 153.—Pseudotuberculosis of mice. Morphology of *Corynebacterium kutscheri* grown on agar. Loeffler's stain. Photomicrograph (×1500). (*From Andrewes, et al.*)

Microscopic examination reveals that the nodules or "tubercles" are composed of bacteria with varying degrees of cellular infiltration. The picture is not one of cellular proliferation. The serosal nodules and membranes consist of bacteria and a small number of cells, but no fibrin. In lesions of longer duration, the appearance is that of a pyogenic abscess with suppuration and necrosis. Bacteria, which are abundant, group themselves into definite colonies in the tissues and particularly on serous surfaces, appearing as masses of interlacing filaments. Thrombi and organisms are found within blood vessels.

Occasionally at autopsy the only lesion is a small abscess at the site of inoculation. Bongert (25) postulated the production of a toxin to explain these instances, and demonstrated that filtrates of broth cultures or heat-killed organisms could cause death in 10 to 14 days without obvious lesions. Topley and Wilson (287) have confirmed this finding and consider that the organism produces an exotoxin which is lethal for mice.

Etiology.—The etiological agent is *Corynebacterium kutscheri* Bergey (22) (*B. pseudotuberculosis murium* Kutscher, *Corynethrix pseudotuberculosis murium* Bongert) (Fig. 153). It is a true diphtheroid, occurring as slender granular rods with some club forms in young cultures, but showing a great

deal of polymorphism in older cultures. It stains irregularly with the aniline dyes, and is gram-positive and non-motile. Growth occurs aerobically on ordinary media and on Loeffler's serum medium at 37°C. Acid is produced from dextrose, sucrose, and maltose but not from galactose, lactose, mannite, and dextrin. Litmus milk is not changed; nitrates are reduced; no indol is formed, and gelatin is not liquefied. Final differentiation of this organism from other members of the group may be made on the basis of its specific pathogenicity for mice. True diphtheria bacilli (*C. diphtheriae*) have not been found in mice (95).

Infections due to related organisms.—Condrea (46) has described an extremely contagious but benign disease which spread through his mouse colony, attacking 200 mice. Only a few deaths occurred from secondary infection. The disease was characterized by the appearance of small, movable nodules in the subcutaneous tissue of the back or thighs. These foci increased in size, became adherent to the skin, and then ulcerated. Yellowish, serous fluid and necrotic caseous material could be expressed from the nodule after removal of the crust. Microscopically, the exudate showed leukocytes and many gram-positive bacilli. The organisms were easily cultured aerobically on media enriched with ascitic fluid or serum, and resembled the diphtheria bacillus in morphology. The organism differed markedly from *C. kutscheri* in its fermentation reactions—acid but no gas was formed in dextrose, levulose, sucrose, maltose, mannose, arabinose, sorbite, dextrin, inulin, and salicin. Experimentally, the disease could be reproduced by subcutaneous or intramuscular injection without generalized infection. Intravenous administration produced a fatal septicemia with localized abscesses in the lungs and kidneys. Rabbits were not susceptible. Condrea classified the organism in the genus *Corynebacterium* and proposed the name "*Corynebacterium muris.*"

A somewhat similar organism was isolated by Holzhausen (101) from white mice injected with the brain emulsion of a dog suspected of being rabid. Paralysis occurred on the second day, followed by death during the course of the next day. The organism apparently produced a septicemia without gross lesions and was readily cultured from the blood and organs. Morphologically, it had the appearance of a diphtheroid which differed from Condrea's organism in fermenting galactose and lactose but not attacking arabinose. It produced hydrogen sulphide and, in litmus milk, acid without coagulation. No exotoxin was detected. The organism has been classified by Bergey (22) as *Corynebacterium murisepticum*. The experimental disease was specific for white and grey mice.

Mouse septicemia (Erysipelothrix).—"Mouse septicemia" is the name given to an infection first reported in 1880 by Koch (132) in mice which had been injected subcutaneously with putrefying blood. Although infrequent, the disease has been encountered both sporadically and epidemically (94, 173, 153, 305, 17, 204, 61) and has been the subject of experimental investigation (82, 139, 300). The etiological agent is *Erysipelothrix muriseptica*.

The most complete description of the natural disease is given by Wayson (305), who studied an epidemic in migrating California meadow mice (*Microtus Californicus estuarensis*) and house mice (*Mus musculus*). The infected animals " . . . sat about with roached backs, roughened pelage, labored breathing, and with eyelids glued together with purulent exudate, and were easily caught by hand."

The gross pathology was that of a septicemia with purulent conjunctivitis and congestion of the subcutaneous vessels producing a deep reddish-pink color in the subcutaneous tissues, particularly about the lymph nodes. Dark red patches of pneumonic infiltration were present in the lungs, with a small amount of effusion in the pleural cavity. The spleen was enlarged and, together with the lymph nodes and liver, was congested and showed occasional tiny white areas of necrosis. Scattered subserous petechiae were noted in the intestinal walls. Organisms were present in large numbers in the blood and viscera. Wayson considered that the infection was spread by cannibalism and by excreta.

The disease may also occur in stock laboratory mice. In performing routine examinations on dead mice from a normal stock, Balfour-Jones (17) noted purulent conjunctivitis, a peculiar gelatinous appearance of the abdominal organs, enlargement of the spleen, and small discrete greyish-white areas about 1 to 2 mm. in diameter in the liver. The lesions appeared as pits on the surface of the liver, and microscopically consisted of round areas of necrosis surrounded by an outer zone of leukocytes. During a 4-month period, 59 of 393 mice showed the above picture—chiefly mice weighing between 12 and 15 grams. The organism isolated reproduced the disease and was identified as an *Erysipelothrix* strain.

Experimentally, the disease may be reproduced by parenteral injection, by oral, dermal, and conjunctival routes, and by inhalation. A septicemia results from parenteral injection, fatal in 2 to 5 days. By other routes the infection progresses more slowly. The first sign of illness is conjunctivitis, at first serous, then purulent, gluing the eyelids together. Lassitude follows; the animal sits with arched back and becomes anorexic and con-

stipated. Respiration decreases in rate, and death occurs almost imperceptibly. The pathological findings are essentially those described above. Mice and rats are susceptible to infection, guinea pigs and rabbits resistant.

Erysipelothrix muriseptica (*B. murisepticus*) is one of three organisms (*E. rhusiopathiae, E. erysipeloides*) which are indistinguishable morphologically, culturally, and serologically. Occasional differences in pathogenicity occur—the murine organism, for example, usually does not infect hogs as does the swine erysipelas strain (*E. rhusiopathiae*)—but are not constant enough to permit classification on that basis. The organisms appear as slender, gram-positive, non-motile rods and as long filaments of threads with irregular thickenings and branching. They are facultative aerobes and grow readily in dew-drop colonies on ordinary agar. In gelatin stab cultures a characteristic "test tube brush" appearance is seen after 3 to 5 days at room temperature—fine threads radiate horizontally into the medium from a central mass of growth along the needle track. No liquefaction is produced. In broth, a slimy viscous growth occurs which settles to the bottom of the tube. The fermentative reactions vary widely, but in general acid is produced in dextrose, lactose, sucrose, maltose, galactose, and raffinose after 48 hours' incubation. Hydrogen sulphide is formed; nitrate reduction is variable. Indol is not produced. Serologically, the organism agglutinates with commercial swine erysipelas serum or antiserum produced with any strain of the group.

The infrequent occurrence of the disease in mice renders its control of little practical importance, but active and passive immunization should be feasible.

Diseases Due to Infection with the Streptobacillus and Pleuropneumonia-like Organisms—Arthritis of Mice

In 1929 Levaditi and Selbie (151) isolated a strain of *Streptobacillus moniliformis* from two mice which had been injected with an emulsion of the brain and spinal cord from an apparently normal mouse. Similar organisms had been isolated previously from human patients in France (148) and in America (*Haverhillia multiformis*) (198). Subsequent work has shown that these organisms are identical with the older *Streptothrix muris ratti*, and the name of *Actinomyces muris* has been proposed by Topley and Wilson (287). In view of common usage, however, the name of *Streptobacillus moniliformis* will be retained here. The organism has been found not only as an inhabitant of the nasopharynx and tissues of apparently

normal rodents, but has also been identified as the etiological agent of sporadic and epidemic illnesses.

The etiological situation in respect to these diseases, however, is not an uncomplicated one. From cultures of *Streptobacillus moniliformis* and from mouse and rat tissues, Klieneberger and her co-workers (125, 126, 127, 72) have isolated a pleuropneumonia-like organism, termed L_1, which alone is relatively avirulent, but in combination with the *Streptobacillus* is markedly pathogenic for mice. The relationship between the two organisms is not clearly established; symbiosis (125, 126) and bacterial variation (48) have both been advanced as explanations. Other pleuro-pneumonia-like organisms, distinct from L_1, have been isolated from diseased mice, and apparently play an etiological role. It therefore seems desirable to discuss together the diseases produced by these two groups of organisms.

Infection with Streptobacillus moniliformis.—This disease, which is primarily an arthritis in the subacute and chronic cases, occurs both sporadically and epidemically (152, 261, 161, 301). The origin of the infection and the factors responsible for epidemic spread remain unknown. Presumably carriers may exist within a stock or may gain access to it from without (wild rodents) and thus serve as the source. The mortality is usually high, but varies in different genetic strains of mice. In one epidemic (161, 301) lasting 4 months, 414 of 650 Simpson-Marsh albino mice succumbed, whereas only 61 of about 300 Little dilute brown (dba) mice died. Death may occur in a few days or at any time within 6 months or longer after infection.

The natural disease.—In the acute form the disease is septicemic in character. Signs of the infection are nonspecific—the animal appears ill, its coat is dull, a semipurulent conjunctival discharge is present, and occlusion of the palpebral fissures may occur. No characteristic changes are found post mortem either grossly or microscopically, but the organism may be cultured from the blood and organs. In blood smears the organisms appear as quite regular bacilli.

A more characteristic clinical picture is presented in the subacute and chronic cases. Polyarthritis, edema and cyanosis of the extremities and tail suggestive of cardiac failure, conjunctivitis, and emaciation are outstanding signs. Occasionally there occurs involvement of the vertebral column resulting in paralysis of the hind legs, ulceration of the feet with serous exudation and crusting but rarely gangrene, enlargement of the axillary and inguinal lymph nodes, keratitis progressing to destruction of the eye, arrested gestation, subcutaneous nodules, submaxillary abscesses, and

enteritis. Deformity and ankylosis of affected joints occur and are visible by roentgenological examination (301) (Figs. 154, 155, 156, 157).

Pathological examination of the viscera reveals marked enlargement of the spleen with numerous, often confluent, areas of necrosis throughout the pulp. Similar lesions are found in the liver, though to a less extent. Con-

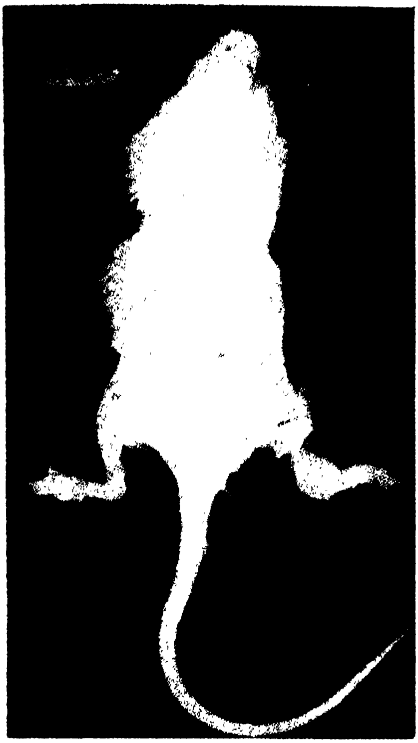


FIG. 154.—Arthritis and enlargement of right ankle joint in a mouse naturally infected with *Streptobacillus moniliformis*. (*From van Rooyen, J. Path. and Bact.*)



FIG. 155.—Infection with *Streptobacillus moniliformis*. Mouse showing edema of legs and tail. (*From van Rooyen.*)

gestion is present in the intestines with enlargement of the lymphatic structures. The kidneys and lungs are not affected. Involvement of the heart is frequent and may vary from a serofibrinous pericarditis to a pancarditis. Microscopically, endocarditis may be evidenced by small vegetations on the valves and endocardium. The myocardium may show numerous foci of inflammation, either acute or undergoing repair. Thrombosis of arterioles and emboli of organisms in the capillaries occur. The articular and osseous lesions vary from acute inflammatory to necrotic or proliferative processes.

In other subacute cases, the picture is that of a fibrinopurulent synovitis with extension of the inflammation into surrounding structures. Organisms may be visualized in thrombi and inflammatory foci as pleomorphic bacilli, filaments, etc. In chronic cases, walled-off necrosis, proliferation of cartilage, or granulations and adhesions may replace the destroyed tissues. Organisms are found extracellularly and within the cytoplasm of large



FIG. 156.—Infection with *Streptobacillus moniliformis*. Three bulbous swellings of tail shown in mouse on left; normal mouse on right. (*From van Rooyen.*)



FIG. 157.—Infection with *Streptobacillus moniliformis*. Mouse showing moist coat, occlusion of palpebral fissure, and paralysis of hind legs. (*From van Rooyen.*)

mononuclear cells. Cultures of blood, organs, and articular fluid yield the organism even in the most chronic cases.

The mode of transmission of the spontaneous disease is not clear. Spread by contact and cohabitation presumably occurs, and bites of infected animals probably transfer the infection. Although organisms may be present in the urine, infection does not appear to be transmitted by contamination of food or water and all attempts to infect animals experimentally by the enteral route have failed (198, 152). Similar but nonpathogenic organisms may be found in the lungs of normal animals and in the dust of hay and straw (112).

The experimental disease.—Types of infection entirely similar to the spontaneous disease may be produced by inoculation of pure cultures. Intravenous or intraperitoneal injection produces the acute septicemic form of the disease; subcutaneous or intra-articular administration, or instillation into the conjunctival sac results in the chronic disease described above. Mice show considerable variation in their susceptibility, not only individually but genetically. Albino strains are more susceptible than the wild brown mouse or hybrid black-coated stock. Other animals in general are relatively resistant to infection.

Etiology.—The organism is a facultative aerobe which grows on liquid or solid media containing 40 per cent or more of serum. Loeffler's medium



FIG. 158.—Film from a culture of *Streptobacillus moniliformis* immediately after isolation. Basic fuchsin. (×900.) (*From van Rooyen.*)

or serum agar is very satisfactory for its isolation. Morphologically, marked pleomorphism occurs in recently isolated cultures; slender gram-negative bacilli, coccoid bodies, and interlacing filaments are present. Large fusiform, oval, or club-shaped swellings may occur at any point in the filaments. After repeated transplants, the morphology becomes more regular and bacillary in form (Fig. 158).

On the surface of solid media, the colonies are of fair size (1 to 2 mm.), greyish, translucent, but not particularly characteristic. Around the colony, often imbedded in the agar, tiny microscopic colonies with dark centers may be found (125, 126, 48). These are the L_1 colonies (referred to above), morphologically characterized by large bodies, granules, and filaments.

Their presence in cultures of the streptobacillus was not confirmed by van Rooyen (301).

In ascitic fluid or serum broth the organism typically produces separate cotton-ball or flake-like colonies which are frequently attached to the sides of the tube but settle down to the bottom if disturbed. A clear supernatant fluid is left. Surface growth does not occur. Filtrates of cultures are not infective (301).

Identification of the organism is made chiefly by the type of growth in liquid media and the morphology. Biochemical reactions are of little help—acid is produced from glucose, salicin, and sometimes lactose and maltose. Serologically, the strains thus far studied are antigenically the same (161, 301).

Differential diagnosis.—Diagnosis of this disease clinically involves differentiation from three diseases, pasteurellosis, mouse pseudotuberculosis, and ectromelia. Animals infected with *Pasteurella* may show a rapidly fatal systemic infection, conjunctivitis, paralysis of the hind limbs, and rarely arthritis, but no edema or cyanosis occurs. Mouse pseudotuberculosis may be differentiated by the absence of conjunctivitis and edema and by the pathological findings. In ectromelia a dry gangrene of the feet and legs is characteristic, paralysis and conjunctivitis are absent, and recovery usually occurs. Final differentiation, however, is made by culture, except in the case of ectromelia where inclusion bodies and the demonstration of a filterable virus establish the diagnosis.

Immunity.—Immunity to the disease occurs naturally, as already pointed out. Animals recovered from the spontaneous disease or injected with heat-killed organisms are resistant to further infection. It is interesting to note that neither infection nor the development of immunity has followed the feeding of cultures (198).

Infection due to pleuropneumonia-like organisms.—Minute pleuropneumonia-like organisms have recently been reported as the etiological agents of experimental disease in mice by investigators both in England (70, 72) and in this country (224, 225, 226, 227, 49, 264, 230). The organisms are of particular importance to those studying viruses because the agents are filterable and do not grow in ordinary culture media. Thus far, no cases of spontaneous illness in mice have been reported, although the micro-organisms have been found in instances of pulmonary disease in rats (128). The appearance of pleuropneumonia-like organisms has followed inoculation of mice for other purposes with such agents as yellow fever virus, lymphocytic choriomeningitis virus, and the toxoplasma, or by

serial intranasal passage of suspensions of mouse lung. Seven distinct strains, termed L_1 to L_7 in accordance with Klieneberger's nomenclature, have been isolated in England, four of which were found in mice. Similarly, five separate types, A, B, C, D, and E of Sabin (227, 230), have been discovered in laboratory mice in America. Illness produced by these agents is important in that it may appear in animals inoculated with other materials and must therefore be recognized. Since the organisms are natural inhabitants of mice, it is probable that under certain conditions they may incite spontaneous disease.

The experimental disease.—The type of experimental disease produced in mice varies with the strain employed and with the route of inoculation. The most striking illness results from intracerebral injection of the L_5 or Type A strain, isolated originally from mice which developed "rolling disease" following inoculation with the viruses of yellow fever or lymphocytic choriomeningitis (70) or with the toxoplasma (224). After an incubation period of 2 or 3 days—occasionally as long as 10 days—signs of illness appear. Some of the animals show little beyond roughening of the fur and irritability; others show a variety of nervous signs and symptoms, often choreiform in type. Characteristically, many afflicted animals tuın in circles with their tails as a fixed axis.

According to Findlay *et al.* (70), approximately 10 per cent of the animals showed the "rolling" phenomenon; the head was rotated slowly, the foreleg raised from the ground, and with a jerk the animal rolled over in one direction for fifty or more revolutions. Death usually followed within 24 hours after rolling began. Three-fourths of the animals died in 2 to 7 days, and of the one-fourth surviving, about half developed acute hydrocephalus 1 to 2 weeks later. No attempt was made to separate the virus of lymphocytic choriomeningitis from the L_5 organisms, but it was found that other strains of pleuropneumonia-like organisms mixed with the virus did not produce the disease. Interestingly enough, animals which survived did not show symptoms of choriomeningitis and were no longer susceptible to that virus—possibly another instance of the "interference phenomenon" (218). Pathologically, an intense inflammatory reaction with polymorphonuclear infiltration was found in the substance of the cerebral cortex, the floor of the lateral ventricles, the choroid plexus, and the meninges, frequently resembling acute abscesses. In cases of hydrocephalus, the ventricles were markedly dilated with a corresponding decrease in thickness of the cortex. Smears stained with Giemsa's stain revealed many extracellular and intracellular granules approximately twice the size of the elementary bodies of

the virus of vaccinia. The L_5 organism isolated from these animals was culturally and immunologically identical with the type A of Sabin, although the production of an exotoxin by the L_5 strain has not yet been established.

Sabin's findings differ from those described above in that the majority of the animals recovered in a few days; some, however, showed a relapse or continued to exhibit choreiform movements for months. Variations in the clinical picture were noted with the age of the mice—no signs occurred in the majority of animals younger than 15 days or older than 2 months, although infection occurred; and arthritis developed in about 30 per cent of mice more than 2 months of age. The characteristic lesion was acute necrosis of the caudal pole of the cerebellum and of the tissues around the lateral ventricles. Cerebellar involvement was absent in animals showing no signs of infection, but periventricular involvement was regularly present.

Intraperitoneal or intrathoracic injection of the L_5 or A strain produces convulsions or other signs of involvement of the central nervous system in 20 to 40 per cent of mice (225). Death usually follows in 17 to 48 hours. At autopsy, lesions are found only in the brain, while organisms are demonstrable in the viscera but not in the blood or brain. The explanation of this finding was revealed by the demonstration of a toxin, which passes through a Seitz filter and, injected intravenously, produces nervous signs within 1 or 2 hours. Most of the animals die in a few hours, but those surviving for longer periods exhibit the same acute degeneration of the cerebellum described above. No organisms can be demonstrated in such animals (225).

Serial intranasal inoculations of an emulsion of lung from a "normal" mouse by the method of blind passage, that is, employing the pulmonic tissue of one animal as the inoculum for the next, may result in a pneumonia apparently due to pleuropneumonia-like organisms after a variable number of passages (264). The infection progresses rapidly with ruffling of the fur, anorexia, weight loss, and dyspnea. Death occurs after 4 or 5 days in about one-third of the mice; if the animals survive for 7 days recovery usually takes place. At autopsy, purple areas of pneumonic consolidation are found involving one or more lobes or an entire lung. Pleuritis may occur. Microscopically, the picture is one of an interstitial pneumonia, congestion, and infiltration chiefly with mononuclear phagocytes. In recovered mice, pneumonic areas may persist for as long as 3 weeks, or a cystic degeneration, similar to that occurring in rats naturally infected with pleuropneumonia-like organisms, may take place. Organisms can be isolated by culture of the emulsified lesions. In addition to the Type A

organisms which produced the infection just described, both Types B and C have been found in pneumonic lungs (227, 103, 104). A conclusive etiological relationship has not yet been established for the pleuropneumonia-like organisms found in the lungs of these animals. Sullivan (263) has found that after six consecutive passages of the organism on killed egg membranes, pneumonic lesions were produced by a single inoculation, which makes probable but does not prove a causal relationship. Further work is necessary to differentiate this type of pneumonia from that caused by viruses (54, 89, 103, 104).

The other pleuropneumonia-like organisms isolated from mice (L_1, L_3, L_6 and Types B, C, D, and E) produced arthritis in a variable number of inoculated animals. The B strains (226) caused a migratory polyarthritis in almost 100 per cent of mice injected intravenously or intraperitoneally. The disease thus produced is chronic, non-fatal, and often results in ankylosis with a pathological picture of proliferation of joint structures. The L_6 strain causes arthritis in about 30 per cent of mice, whereas the L_1 strain only occasionally affects the joint (72). Dienes and Sullivan (50), however, have not succeeded in producing infection in mice with the L_1 organism. Production of toxin by these strains has not been demonstrated.

The experimental disease (72) produced in mice by inoculation of the L_7 strain, obtained from rats showing polyarthritis, is of considerable interest because of its similarity to that described above as caused by *Streptobacillus moniliformis*. In some animals swelling of the tibiotarsal joint, edema of the subcutaneous tissues, and death followed inoculation of the foot pad with cultures mixed with agar. With intracerebral inoculation, weakness of the hind legs, hunched back, tremors, turning in circles, and occasionally conjunctivitis developed. Intravenous and intraperitoneal administration resulted in pleural or peritoneal exudation and arthritis in animals surviving 48 hours or longer. Intranasal instillation produced pneumonia in 5 to 8 days. In smears of animal tissues rings, granules, and comma-shaped structures were seen. In spite of the fact that the L_1 strain, apparently associated with *Streptobacillus moniliformis*, and the L_7 strain are distinct and separate organisms, the points of similarity between the two diseases provide an adequate basis for etiological confusion.

In general, animals other than the mouse are resistant to infection with mouse strains, and even among mice considerable variation in susceptibility is found between individuals and breeds. Mice surviving experimental inoculation are resistant to reinjection with the same strain although no humoral antibodies are demonstrable.

The carrier incidence in stock mice has not yet been adequately determined, but may be as high as 40 to 80 per cent. This state probably develops after birth from contact with the mother and persists throughout life. Organisms have been cultured from the conjunctiva, nasopharynx, lungs, and brain, but not from the blood, liver, spleen, kidneys, or intestinal contents (72, 230). Natural antibodies have not been demonstrated in such animals.

Etiology.—Pleuropneumonia-like organisms may be cultivated on special agar media* directly from the lesions in mice, or from the conjunctiva and nasopharynx, although they are more difficult to obtain from tissues of normal animals. After 24 to 48 hours' incubation tiny colonies, 20 to 100 μ in diameter, may be seen under the microscope. Frequently they have clear margins and dark centers and consist of granules, globules, and fine filaments. Examination may most simply be made by staining the colonies directly in the agar (48) with methylene blue or azure II, although other special techniques have been employed (125, 126).

Growth of the organisms ordinarily occurs in liquid media as a faint opalescence appearing after 36 to 48 hours of incubation at 37°C. Meat infusion or nutrient broth containing 30 per cent ascitic fluid or sterile serum is satisfactory. Addition of 0.5 per cent glucose is apparently advantageous with some strains. Dark field examination reveals tiny granules and occasionally globules and small filaments. On subculture to solid media, the characteristic microscopic colonies appear. The various strains may be differentiated to some extent by culture, but more satisfactorily by immunological methods. Sabin (229) has stated that the members of the pleuropneumonia group of organisms found in mice are immunologically and pathogenetically different from those found in the rat.

The organisms are approximately 250 to 300 mμ in size, as determined by filtration through gradocol membranes (225), and pass a Berkefeld V filter. They are inactivated at 45°C. for 15 minutes but remain infective for more than 30 days in 50 per cent buffered glycerin and for months if frozen and dried by the Flosdorf-Mudd lyophile method. Toxin production is thus far demonstrable with only one strain (Type A). The toxin appears early during growth, lasts only about 2 days after its appearance, is inacti-

* A satisfactory medium may be prepared as follows: 5 per cent defibrinated blood is added to 2 per cent meat infusion agar (pH 7.6 to 8.0), the mixture brought to the boiling point, immediately cooled to about 50°C., and the clear supernatant removed after the coagulated blood has settled out. To this is added about 30 to 40 per cent ascitic fluid before pouring into Petri plates.

vated at 50°C. for 30 minutes, and is antigenic, producing an antitoxin which neutralizes its action (225, 227). Antisera specific for the various strains may be produced in rabbits. Organic gold preparations are bactericidal for these organisms *in vitro* and are highly active in preventing experimental infection (71).

Epidemic Pneumonia in Mice

Pneumonic lesions in mice may be found in several of the bacterial diseases already described and in certain of the virus diseases. In addition, apparently distinct epidemic respiratory infections associated with at least three other bacteria have been reported. In each instance pneumonic involvement has been a prominent part of the clinical and pathological picture. A brief description of these epidemics follows.

Infection associated with Brucella bronchiseptica.—In 1920, Keegan (118) reported an epidemic which occurred in an animal room containing 150 mice and 86 guinea pigs. The disease appeared first in the mice as a prolonged illness causing death in a few of the animals. It was characterized by a profuse purulent conjunctivitis with swelling of the eyelids and desquamation and depilation of the surrounding skin, roughening of the fur, nasal discharge, and occasionally death. A few weeks later the condition increased in severity, with the additional signs of rapid, labored breathing and weight loss. Mice killed at this time showed a firm, greyish-white, lobular consolidation of one or more lobes without pleuritis. The bronchi were dilated and filled with thick purulent exudate. Microscopic sections revealed purulent bronchitis and bronchopneumonia. The bronchi were filled with polymorphonuclear exudate; the mucosa was thickened or desquamated in some areas and mononuclear infiltration was present in the walls and about the bronchi and blood vessels. Alveolar lesions consisted of areas of polymorphonuclear exudate and partial or complete atelectasis. During the latter part of the epidemic some of the animals succumbed rapidly instead of after a prolonged course. At autopsy, hemorrhagic lesions were present in the lungs, which presented a microscopic picture varying from marked capillary engorgement and serous alveolar exudation to frank hemorrhage. Fifty of the 150 mice developed signs of illness. The mortality was low but the incidence high since many mice, apparently normal, showed pulmonary lesions when killed. Infection in the guinea pigs developed late in the course of the epidemic, appearing first in those cages closest to the mice.

Cultures from 6 of 25 mice autopsied and from 12 of 15 guinea pigs showed *Brucella bronchiseptica* (*B. bronchisepticus*). This organism is a gram-negative, motile, cocco-bacillus which grows on ordinary media but does not produce acid or gas from carbohydrates. An alkaline reaction is produced in litmus milk, and ammonia is formed from urea and asparagin. Neither hydrogen sulphide nor indol is formed.

As Keegan points out, the low incidence of positive cultures in mice may have been due to the fact that tracheal cultures were not made. On the other hand, *B. bronchiseptica* is not a highly pathogenic organism and is frequently associated with other agents such as viruses—in canine distemper, for example.

A pathologically similar condition which occurred spontaneously in chronic form in approximately 3 per cent of stock mice has been reported by Branch and Stillman (28, 29). No attempt was made to isolate the etiological agent. The chief lesion is one of pulmonic consolidation occurring irregularly in the various lobes but most often involving the right medial lobe. Lesions may be multiple or may involve the whole of a single lobe. Early in the disease the affected areas appear red, firm, dry, and hepatized; later they become greyish, gelatinous, and translucent in appearance, and the surface is irregularly granular and puckered. Pleurisy is rare. The peribronchial lymph nodes are enlarged and the lesions tend to follow and persist in the peribronchial and perivascular tissue. Microscopically, the bronchial exudate contains many polymorphonuclear leukocytes, whereas the areas of alveolar consolidation consist largely of mononuclear cells. Fibrin is rarely found. Focal areas of necrosis are occasionally found.

The same disease occurs sporadically in the stock animals at the Jackson Memorial Laboratory. It progresses slowly and is recognized in the late stages by failure of the animals to thrive and breed, roughening of the fur, weight loss, rapid labored breathing, and finally death. Investigations are being carried out to determine the etiological agent and the possible relationship of *B. bronchiseptica*.

Infection due to a Friedländer-like bacillus.—During the course of his investigations on experimental epidemics of mouse typhoid, Webster (318, 319) encountered two outbreaks of respiratory infection due to a Friedländer-like bacillus. The disease first appeared in the summer (August), recurred in successive waves of decreasing severity, and disappeared in the spring. The morbidity and mortality were high.

The clinical manifestations varied; some animals developed pulmonary involvement, others septicemia, nasal infection, or the carrier state. The

incubation period was about 48 hours. Transmission occurred via the nasal passages by contact. When carriers were added to a group of 100 or more mice, some died within 5 days, 50 to 70 per cent succumbed within 2 weeks, some contracted the disease and recovered, and others were refractory. Organisms were not found in the bedding, food, or feces. Intranasal inoculation reproduced the pulmonic lesions with as few as 200 to 600 organisms.

At autopsy, subserous petechial hemorrhages characteristic of septicemia and bilateral pneumonia were noted. The lungs were red and moist, on section contained little air, and the fluid expressed from the cut surface was viscid and stringy. Pleurisy was frequently present. The microscopic picture varied with the duration of the lesion from interstitial congestion, hemorrhage, edema, and serous alveolar exudate in the early lesions to a cellular exudate of polymorphonuclear cells filling the alveoli. A fibrinous and cellular exudate covered the pleural surfaces. In general, the pathology was similar to that following experimental infection of mice with Friedländer's bacillus (29).

Cultures of the nasal passages, lungs, and blood yielded a large, gram-negative, capsulated bacillus which was morphologically and culturally indistinguishable from Friedländer's bacillus (*Klebsiella pneumoniae*). Better growth occurred at 23°C. than at 37°C. The organism, however, was entirely distinct antigenically from five known types of Friedländer's bacillus.

Infection due to an organism resembling the influenza bacillus.—Kairies and Schwartzer (116) have described a sporadic and epidemic disease due to an organism resembling the influenza bacillus. Sporadically ill animals showed weakness, shaggy coats, adherent eyelids, frequent abscesses on the head, rapid respirations, and bronchopneumonic lesions in the lungs. During the epidemic in which 2 to 4 mice of a stock of 500 died daily, diarrhea was an additional feature. Leukopenia resulted from infection with the organism, the leukocyte counts varying from 1200 to 4000 with a decrease in lymphocytes and an increase in segmented and stab forms. Cultures of the pharynx, nose, eyes, abscesses, heart's blood, and lungs of diseased mice yielded a tiny gram-negative cocco-bacillus showing bipolar staining in young cultures but involution and thread forms in old cultures. Approximately 35 to 40 per cent of healthy animals were found by culture to harbor the bacillus. Occasionally a hemolytic streptococcus was recovered with the cocco-bacillary organisms from all parts of the body; rarely the streptococcus alone was found in the respiratory tract. Although not strongly hemoglobinophilic, the bacillus morphologically and culturally was almost

impossible to distinguish from Pfeiffer's bacillus and the name "*Bacterium influenzae murium*" was accordingly proposed. Certain aspects of the spontaneous illness, as well as the hemorrhagic exudation produced by experimental inoculation, resemble mouse pasteurellosis (hemorrhagic septicemia), from which it should be differentiated. In view of the fact that some cultures in liquid media contained tiny bodies passing a membrane with a pore diameter of 400 to 600 mμ, a relationship may exist between these forms and the pleuropneumonia-like organisms or the cocco-bacilliform bodies of infectious catarrh of mice (178).

Spontaneous pneumonia due to the pneumococcus has not been reported, although the isolation of this organism from mice has been claimed (194). A partial explanation may be found in experimental attempts to produce pneumonic lesions. Following inhalation of virulent Type I pneumococci, which killed rapidly by other routes of inoculation, the organisms were found in the lungs only up to 3 hours after administration (256). Pneumonia did not result unless the animals were partially immune (258, 259) nor did systemic infection follow (139, 256) unless the animals were intoxicated with alcohol (257). Experimental pneumonia readily occurred following inhalation of virulent hemolytic streptococci and Friedländer's bacillus (256, 29). Later work, however, has demonstrated that certain strains of pneumococci, inoculated intranasally, produced in some mice a fatal respiratory and general infection with septicemia, pneumonia, pleurisy, empyema, pericarditis, and cervical lymphadenitis (325). By balancing the virulence of the organism and the resistance of the mice, it was possible to produce pneumonia with a consistency which permitted study of the pathology and pathogenesis of the infection (213, 214).

Cultures of the lungs of normal mice (112) have yielded organisms of the streptothrix type (*Streptobacillus*), *B. subtilis*, and various kinds of cocci. Similar organisms were found in the dust from hay and straw, and by withholding such substances the number of contaminating organisms was reduced. These bacteria were non-pathogenic when injected subcutaneously, however, and probably are of little importance in the production of disease.

Control measures to be undertaken in the event of an outbreak of respiratory infection are simply those of isolation and the usual precautionary procedures to prevent spread. In an unusually valuable stock, chemotherapy with one of the sulfonamide drugs (sulfanilamide, sulfapyridine, or sulfathiazole) might be attempted, although no information is available regarding the efficacy of any of them in these infections.

Infectious Catarrh of Mice

This disease has been described by Nelson (178, 179, 180) as an epidemic occurring in an isolated group of Swiss mice and their offspring, together totaling approximately 800 mice. The condition was chronic in nature, but spread so widely through the colony that after 10 months all but 75 of the animals were killed. During the next 11 months 72 of the 75 mice died—a mortality of 96 per cent. An endemic type of the disease has been noted in one colony in which only sporadic cases were observed.

Signs of illness appeared after an incubation period of 10 or more days. Intermittent "chattering" was commonly the first evidence of infection



FIG. 159.—Infectious catarrh of mice; characteristic posture, ruffing of hair, and abrasions about the ear. (*From Nelson, J. Exp. Med.*)

and became more constant as the disease progressed. This sound is distinctive, apparently is produced in the lower part of the respiratory tract, and resembles that made by rapid, gentle clicking of the teeth. Rhinitis, found at autopsy but not associated with visible nasal discharge, appeared early, as did snuffling. Some of the animals showed ruffled fur, rapid, shallow respiration, weight loss, and death 3 to 5 weeks after the appearance of chattering. Others appeared to be normal, except for chattering, for many weeks but eventually developed considerable loss of hair and scabby skin, occasionally marginal necrosis of the ear, and terminal respiratory difficulty (Fig. 159). True conjunctivitis infrequently occurred. The disease was invariably transmissible to normal animals by direct contact and by intranasal instillation of exudate from the respiratory tract and middle ears of naturally infected mice.

Postmorten examination of 45 animals, either naturally or experimentally infected, revealed rhinitis in 43, otitis media in 43, and pneumonia in 35. A thick, semi-fluid, mucopurulent exudate was present in the nasal passages. Stained films of the discharge showed the "coccobacilliform" bodies to be described later, many leukocytes, and mucus strands. The

tympanic cavity was filled with a copious, purulent exudate containing many white blood cells. The pneumonia, which was progressive and finally resulted in death of the animal, was usually lobar in distribution. The involved area was consolidated, contracted, and red, grey, or mottled in color. In advanced cases, all lobes were sometimes involved. Bronchial exudate of polymorphonuclear cells, secondary alveolar extension as indicated by leukocytes, erythrocytes, large mononuclear cells, and fluid in the alveoli, and hyperplasia of the peribronchial lymphoid tissue were the chief features microscopically.



FIG. 160.—Infectious catarrh of mice; scattered extracellular coccobacilliform bodies in nasal exudate. Gram stain. (×920.) (*From Nelson.*)

In stained films of exudate from the nares, middle ears, and lungs of diseased animals were found small gram-negative cells, termed "coccobacilliform bodies" because of their similarity to those of fowl coryza (Fig. 160). The organisms were generally spherical, but rod-shaped cells and ring forms were seen. They occurred singly, in pairs, and in loose clumps. Although predominantly extracellular, they were also found in polymorphonuclear leukocytes and epithelial cells. Attempts to cultivate the organism on "ordinary nutrient media enriched with blood" were unsuccessful, although growth occurred in tissue culture and in the supernatant fluid of tissue culture media. In several instances, pure cultures of the coccobacilliform bodies were obtained, especially from the middle ear; in others, the organism was found in association with staphylococci, streptococci, or an unidentified short, non-motile gram-negative bacillus, termed the "X" bacillus.

The average diameter of the coccobacilliform bodies by direct microscopy was between 0.3 and 0.4 μ. The bodies passed through a collodion membrane with an average pore size of 640 mμ, which indicates an average particle diameter of 480 mμ. Such filtration, however, did not separate the organism from the X bacillus.

The etiological relationship of the coccobacilliform bodies to mouse catarrh seems to be established since the disease was reproduced by pure

cultures after as many as twelve subcultures in tissue medium. Filtration through Berkefeld V candles apparently removed the organism and excludes the possibility of a filterable virus, since filtrates were not infective. The X bacillus was not pathogenic.

The possibility has not been excluded that this disease is related to that described by Kairies and Schwartzer (116) as being due to an influenza-like bacillus. It seems probable, however, that the mouse catarrh is a distinct entity. The similarity of the coccobacilliform bodies to the pleuropneumonia-like organisms is striking and warrants further investigation.

Pyogenic Infections, Botriomycosis

Pyemic and suppurative lesions are frequent in mice as well as in other laboratory animals. From subcutaneous abscesses may be cultured such organisms as *Staphylococcus aureus* or *albus*, *Gaffkya tetragena* (*Micrococcus tetragenus*), hemolytic or non-hemolytic streptococci, rarely *Bacillus pyocyaneus*, and others of less importance. The lesions may arise at the site of incarcerated worm rests and show a variable bacterial flora (299). Abscesses in the heart and lungs, from which pure cultures of both white and yellow micrococci were usually obtained, have been found in 3 per cent of 12,000 autopsies on mice (299). Certain of the cocci—*Gaffkya tetragena*, for example—are very pathogenic for white mice, septicemia and death occurring within 2 or 3 days after experimental inoculation by almost any route (73).

Abscesses about the head and neck have been observed not infrequently in the mice at the Jackson Memorial Laboratory and have been found in one instance by Tyzzer (297). Pathologically, the condition resembles that termed "botriomycosis." The lesions are walled off and composed of areas of granulation tissue enmeshed in fibrous strands with numerous areas of dense polymorphonuclear exudation. Scattered through the lesions are granules, irregular in outline, and surrounded usually by polymorphonuclear exudate. Many show a rather homogenous outer rim, staining pale blue with hemotoxylin and eosin, while the remainder of the granule varies from deep blue to pink. Occasional club-like excrescences may be seen. The structure of the granules may be granular and amorphous, or may suggest a central cellular appearance, likened by some investigators to cocci embedded in zooglial substance (287, p. 1181). Although the disease in mice has not been adequately investigated, considerable evidence is accumulating which indicates that in other animals the lesions are due to staphylococci (287, pp. 1180–81; 124, 123).

Kutschera (136) observed a spontaneous epidemic in white mice due to a streptococcus, although a staphylococcus was present as well in many animals. The affected animals appeared ill, their eyelids were adherent, hair roughened, and breathing rapid. At autopsy the spleen was enlarged to three or four times normal size and studded with yellowish, pin-head sized abscesses. Of 30 mice studied bacteriologically, most showed a double infection with streptococci and staphylococci. Organisms were seen in smears of the liver, kidney, spleen, heart, and bone marrow. Experimental inoculations of normal mice with organ suspensions of infected animals produced death in 1 to 2 days, the findings resembling those of the spontaneous disease without abscesses. Subcutaneous inoculation with a pure culture of the streptococcus resulted in death in 3 days. A local abscess was formed at the injection site, about which the tissues were hemorrhagic; punctiform hemorrhages were present in the peritoneum, intestines, and testicles, and the spleen was swollen. Similar experimental infections with streptococci have been obtained by other workers (139, 300).

Although such epidemics are rare, apparently normal mice may harbor streptococci (341). Cultures of the blood of 35 white mice were positive for streptococci in two instances. After injection with sterile milk, adrenalin, or plague vaccine, 6 of 35 mice showed streptococci by blood culture. The strains obtained were not identical in their cultural reactions and only one produced hemolysis, although all were gram-positive cocci, growing in long chains.

Infection with Bacillus Piliformis

A highly fatal bacterial disease in Japanese waltzing mice has been reported by Tyzzer (295). The disease spread among this inbred stock in epidemic fashion, affected a few hybrids of the first and second filial generations (F_1 and F_2), but did not involve the common laboratory mice. It presumably originated from the common mouse during cross-breeding experiments, although the organism was not found in stained sections of the intestines of many laboratory and wild mice.

Signs of infection appeared 24 to 48 hours before death and consisted of roughened fur, ataxia, and watery or slimy diarrhea. In young animals the disease was more acute, with diarrhea the prominent feature. Diagnosis could be made in some animals by removing the fur from the abdomen and viewing the lesions on the ventral surface of the liver through the transparent abdominal wall. The time of death varied from 6 to 44 days after exposure, with an average of 10 to 20 days.

At autopsy the only gross lesions were found in the liver, which was enlarged and contained a varying number of opalescent, grey or yellowish nodules. These nodules were usually discrete, varying from less than 0.5 mm. to more than 2.0 mm. in diameter (Fig. 161). Microscopically, the lesions were situated in close proximity to the portal vein, suggesting an embolic distribution. They consisted of necrotic tissue with an extensive peripheral polymorphonuclear infiltration. Hepatic cells about the lesions contained many long, slender bacilli, lying roughly parallel to one another, each organism separated from the adjoining one (Fig. 162). Numerous spores were present as well as vegetative forms apparently undergoing sporulation. Organisms were frequently present in the gall bladder and bile. Although no visible lesions were present in the alimentary tract, microscopic sections of the cecum and first portion of the large intestine revealed many bacilli and spores within epithelial cells, phagocytes, lymphatics, and in the depths of the glands (Fig. 163). Almost no host reaction was present.

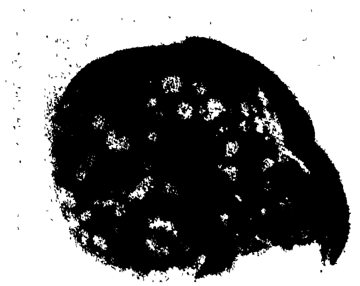


FIG. 161.—*B. piliformis* infection in mice; gross appearance of lesions in liver. (*From Tyzzer, J. Med. Res.*)

The organism was a slender, non-motile, non-acid fast, gram-negative bacillus, showing considerable pleomorphism. Some organisms presented a granular, band-like appearance. Spores were situated subterminally. One attempt to demonstrate the heat resistance of the spores was inconclusive, but a contaminated cage remained infective after one year at room temperature. All attempts to grow the organism in pure culture on enriched culture media failed, except for one occasion when it grew briefly in symbiosis with a streptococcus.

Infection could be best produced experimentally by contact of susceptible mice with diseased ones or with a contaminated cage, and by ingestion of infected tissue or intestinal contents. Common laboratory mice, rabbits, and guinea pigs were resistant. Intravenous injection of waltzing mice with large doses produced the typical liver lesions and death, but minimal lesions and immunity followed small doses. Intraperitoneal and subcutaneous administration did not result in systemic disease. On the basis of these findings, together with the pathological picture, Tyzzer postulated that the fatal disease was produced by a secondary embolic

invasion of the liver following primary infection of the gastro-intestinal tract.

This disease is particularly interesting because of its limited host susceptibility. Further study from the point of view of heredity (90) has

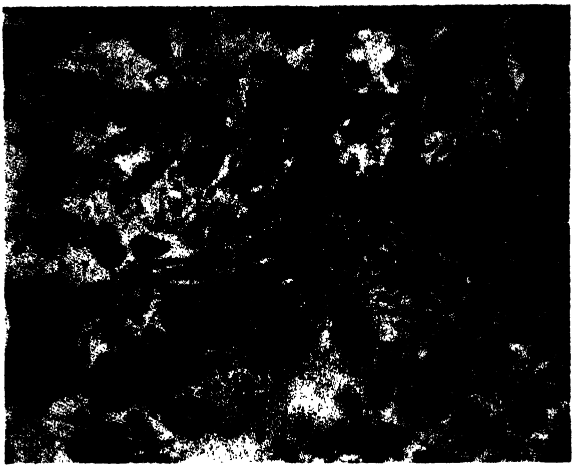


FIG. 162.—Banded bacilli at the periphery of a liver lesion in a mouse dying of *B. piliformis* infection. Stained section (×1400). (*From Tyzzer.*)



FIG. 163.—Smear of intestinal epithelium from a mouse dying of *B. piliformis* infection. Note intracellular bacilli having the appearance of spore-formation. Spore stain. (×1400.) (*From Tyzzer.*)

indicated that predisposition to infection is independent of the waltzing factor, the dominant white factor, and sex. The condition, moreover, may become of greater practical importance, since Tyzzer (297) has recently found it in highly inbred stocks used for cancer studies.

Van Rooyen (301) has inferred that this disease may be the same as that produced by *Streptobacillus moniliformis*. Apart from clinical differ-

ences, it is difficult to reconcile the identity of the two organisms because of the morphological appearance of the spores of *B. piliformis*, the failure of this organism to grow in serum-enriched media on numerous attempts, and the persistence of infective material in a contaminated cage for a year.

Infection Due to Bartonella, Eperythrozoon and Grahamella

Three types of organisms which parasitize red blood cells have been found in mice, as well as in other rodents. None produces obvious clinical disease under natural conditions. Infection with two of them, *Bartonella* and *Eperythrozoon*, remains latent until manifested by splenectomy, exposure to x-ray, or infection with an unrelated agent. The grahamellae, on the other hand, are less notably affected by such events. In Table 4 are summarized the chief characteristics of these three organisms.

The exact classification of these organisms has been subject to considerable doubt and disagreement. Bartonellae and eperythrozoa have been thought by some to be protozoa, by others to be bacteria or *Rickettsia*-like organisms. Certain authors, in addition, have considered the grahamellae to be basophilic granulations in the erythrocytes. Although final classification cannot yet be made, it seems probable that the organisms are closely related to bacteria in view of their morphology and behavior. An excellent review of bartonella and eperythrozoon infections up to 1935 may be found in the monograph by Weinman (331).

Infection with Bartonella.—Bartonellosis as a disease is perhaps best known in man (Oroya fever) and in the rat (infectious or "pernicious" anemia of rats), but it occurs in many other species of animals, especially in small rodents (164, 331, 333). Bartonellae or bartonella-like organisms have been reported in splenectomized white mice (185, 238, 171, 239, 63, 121, 167, 60), white-footed deer mice, *Peromyscus leucopus* (296), and wood mice, *Mus sylvaticus* (34). Infection has also occurred following the inoculation of mice with trypanosomes (164, 156), although it is not clear whether the strains so obtained had their origin in the mouse or were introduced with the trypanosomes. In spite of the fact that some investigators (303, 157, 158, 155) have apparently found spontaneous infection in mice with considerable consistency, the condition must be rare, if not absent, in many mouse stocks. In a combined series of over 100 splenectomized mice (165, 2, 302, 298), no bartonellae were found.

The natural disease.—No instances of spontaneous clinical disease have been reported in mice, although on rare occasions bartonellae may be seen in the blood of naturally infected animals (171). Following splenectomy,

however, latent infections become apparent, and organisms may be seen in great numbers in the blood after an incubation period varying from 1 to 4 days. The organisms increase in number for 5 or 6 days, then gradually

Table 4

COMPARISON OF *Bartonella*, *Eperythrozoon*, AND *Grahamella**

	Bartonella	*Eperythrozoon*	*Grahamella*
Morphology	Bacillary and coccoid; occasionally ring-like	Delicate rings or disks; fine short rods	Coarser, bacillary; no coccoid forms
Staining:			
Giemsa	Red to reddish-blue; less intense	Bluish-red or violet; faintly except for organisms applied to margins of erythrocytes	Blue; more intense
Other aniline dyes	Poorly, if at all	Poorly, if at all	More easily stained
Location	Often epiglobular or free	Epiglobular or free; usually a preference for polychromatophilic cells	Endoglobular; uniformly spaced in cells
Number of organisms on or in affected cells	Few	Numerous	Few or numerous
Effect of splenectomy	Latent infections become apparent	Latent infections become apparent	Without marked effect
Chemotherapy with arsenical compounds	Tends to sterilize	Tends to sterilize	No effect
Natural transmission	Arthropod vector	Arthropod vector	Unknown
Pathogenicity	Pathogenic	Usually non-pathogenic	Non-pathogenic
Cultivation on artificial media	Sometimes successful	Not proved	Not yet determined

* Modified from Bruynoghe and Vassiliadis (34) and Tyzzer and Weinman (298).

disappear until at the end of 3 weeks they may be found only with difficulty (238, 171, 158). Smears of the blood at the height of the infection reveal organisms in connection with a relatively high proportion of the

erythrocytes. The organisms are few in number per red blood cell and appear to be on or possibly in some instances in the cell itself. A few may be found at times free in the plasma, possibly released by destruction of infected erythrocytes. Ordinarily, splenectomized mice show no signs of infection (238, 171), although some animals develop anemia (303, 155, 167). Pathological examination of splenectomized mice reveals little beyond possible hyperplasia of endothelial cells and foci of lymphocytes in the liver (55). Carrier mice may show some degree of splenomegaly which, however, may occur in the absence of infection of this type. The manner of spread in mice has not yet been established. Arthropods may be the vectors since in rats the flea (*Haematopinus spinulosis*) can transmit the infection (64, 331).

Mixed infections with bartonella and *Eperythrozoon coccoides* may follow splenectomy (238, 157, 158, 121, 60). Lwoff and Vaucel (157, 158) believe that under these conditions the virulence of the Bartonella may be increased.

The administration of arsenicals to both rats and mice is effective in eradicating latent bartonellosis or in treating the more acute infection which follows splenectomy. Dosages recommended (34, 122) are approximately 12.5 mg. of neosalvarsan or 100 mg. of tryparsamide per 100 grams of body weight. A combination of arsenic and antimony (Bayer's "sdt 386") is stated to be particularly efficacious (122). Sulfanilamide has been found to be unsatisfactory in the rat disease (65). Domagk and Kikuth (55) have emphasized that the effective dose of the chemotherapeutic agent must not injure the reticulo-endothelial system if the results are to be satisfactory.

The experimental disease.—Numerous attempts have been made to transmit the mouse strain of bartonella to both normal and splenectomized mice free from the disease, with varying degrees of success. In some instances, transmission has failed (185, 121, 60); in others, massive and at times fatal infection with anemia has resulted even in normal mice (156, 60). The work of Lwoff, Provost, and Vaucel (156, 303, 158, 155) is of some interest in this regard. A non-splenectomized mouse, inoculated with *Trypanosoma cruzi*, developed a bartonella infection, which was then transferred successively in normal mice by inoculation of blood, and was ultimately separated from the trypanosome. The trypanosome had been passed through two rats and one dog before inoculation into the mouse. None of the mice was resistant to the bartonella infection. The organisms appeared usually on the second day, increased in number until the fifth or sixth day, and disappeared about the ninth day. Anemia and splenomegaly were the only significant pathological changes reported. No recurrences

were noted even in those animals in which a fatal infection with the trypanosomes subsequently developed. Inoculation of rats produced an infection of varying severity, but not all rats were susceptible. The organism was cultivated in Noguchi's medium (184), and was termed "virus spontané." A second strain of bartonella, termed "virus provoqué," was obtained following splenectomy of normal mice. The clinical course of the two infections was similar, and no morphological differences were noted in the two strains. Other differences were described, however. The "virus provoqué" was not cultivated. It did not infect normal mice (non-splenectomized) or splenectomized mice which had recovered from the homologous infection, although the latter animals were susceptible to the "virus spontané." Mice, recovered from the "virus spontané," acquired the "virus provoqué" after spelenectomy without alteration in the incubation period or duration of the infection. On the basis of these data, the two strains were considered to be different immunologically.

Evaluation of this work is difficult. The possibility exists that the "virus spontané" may originally have been derived from the rat. Normal mice are susceptible to *Bartonella muris* of the rat (238, 1), and although many authors (331) consider the rat and mouse strains to be identical, definite differences have been reported (238, 167). In favor of the mouse origin of the "virus spontané" is the fact that a similar strain of high virulence was obtained from a splenectomized mouse having a mixed infection with bartonella and *Eperythrozoon coccoides* (157, 158). Kikuth (121), however, encountered a similar mixed infection but was able to transmit only the eperythrozoon to other splenectomized mice.

As already mentioned, *Bartonella muris* of the rat may be transmitted to mice by inoculation of infected rats' blood. Although many animals show no signs of infection (122), a fatal anemia may develop both in normal and in splenectomized animals (238, 1, 2, 239, 167, 3). Repeated passage of the organism in young normal mice may apparently increase its virulence until most of the animals succumb from the infection (1). In such animals all the erythrocytes may be infected by the fourth or fifth day and the number of red blood cells may fall from 9,000,000 per cu. mm. at the time of infection to 1,000,000 per cu. mm. at the time of death on the fifth or seventh day. Hemoglobinuria, common in rats, rarely occurs in mice. In animals which recover, the destruction of the organisms is sudden, constituting a crisis in which the number of infected cells falls from 100 per cent to less than 1 per cent within 24 hours. The spleen is uniformly enlarged (three to four times normal size), and in recovered animals there is

extensive phagocytosis of red blood cells by the pulp cells. In splenectomized mice (2) the course is generally more acute than in normal mice, but varies with the age of the animal. In those younger than 3 months, infection is chronic for a period as long as 4 months, with intermissions and relapses. The red cell count may fall to approximately 2,000,000 per cu. mm., and mononucleosis up to 18 per cent may occur. Most of the animals recover. On the other hand, infection in splenectomized mice 6 months or more of age is usually acute and terminates fatally from 3 to 12 days after *B. muris* appears in the blood.

The function of the spleen in relation to immunity in bartonellosis is of considerable interest and has been the subject of much investigation in rats (75, 76, 77, 64, 166, 4, 332), but a satisfactory explanation has not yet been obtained. Weinman (332) has shown that the anemia has no apparent relationship to immune bodies in the serum, but is hemolytic in type and due to direct action of the organism on the erythrocytes. In mice, as in rats, the spleen seems to offer some barrier to infection, but here also its role in immunity is not clear. By partial ablation, Adler (2) has demonstrated that about 30 per cent of the spleen is sufficient to control infection. Repeated injections of *B. muris* cause splenomegaly, but an immunity persisting indefinitely after splenectomy does not result. It seems likely that the immunological aspects of this disease will not be clarified until cultural methods are employed and larger numbers of virulent organisms are used for immunization.

Tyzzer (296) has recently demonstrated that the natural bartonella infection in white-footed deer mice (*Peromyscus leucopus*) can be transmitted to splenectomized normal white mice. A severe and occasionally fatal anemia results. If the splenectomized mice suffer from infection with *Eperythrozoon coccoides*, however, the bartonella infection is prevented from developing or suppressed if already established. On the other hand, the mouse eperythrozoon may be transmitted to the splenectomized vole (*Microtus pennsylvanicus*), but does not interfere with natural bartonellosis in this animal. Such phenomena of interference, first noted with viruses, have as yet been observed in relatively few instances and the mechanism is not known. The phenomenon must be kept in mind, however, since, as Tyzzer points out, experimental results may be altered by the presence of unrecognized infection.

Etiology.—The bartonellae of rodent origin are small, pleomorphic, gram-negative bacteria which vary in width from 0.1 to 0.5 μ and in length from 0.5 to 2.0 μ. Variations from coccoid to bacillary form may be seen

(185, 34, 122). Ring forms occur in certain species, as well as long rods segmented along their axes in a manner suggestive of division. Cultivation has been successful on Noguchi's leptospira medium and on other media (184, 158, 155, 142, 143, 304, 331, 333) after a period of 1 to 2 weeks of incubation. The optimum temperature is between 25° and 28°C. Cultivated strains may be motile. Growth has also been obtained in egg embryos (304).

In blood smears colored by Giemsa's method the organisms stain a light reddish-blue color. They appear to be on or within the red blood cells (Fig. 164). At the height of the infection, the majority of the cells contain



FIG. 164.—Bartonella in the peripheral blood of a spontaneously infected, splenectomized white mouse. (*From Schilling.*)

organisms, although the number of organisms on a given cell is usually small—from 1 to 10. Placement on the margin of the erythrocytes is common. Organisms have not been demonstrated in endothelial cells apart from those present in phagocytized erythrocytes. The strains found in mice are reported (238, 167) to be smaller and finer than the rat strains, with a greater tendency to the formation of long, thin, bacillary forms.

Schilling (238) has proposed the name *Bartonella muris musculi* for the mouse organism to differentiate it from *B. muris* (*B. muris ratti*) of the rat. Since such nomenclature becomes cumbersome and because of definite differences in the human and animal bartonelloses, Tyzzer and Weinman (298) have proposed two genera for bartonella organisms: *Bartonella*, type species *B. bacilliformis*, to include bartonella which multiply within cells (vascular endothelium) other than erythrocytes and which produce wart-like or nodular cutaneous eruptions, and *Haemobartonella*, type species *H. muris*, to include bartonellae in which there is no demonstrable multiplication outside the blood and which do not produce cutaneous eruptions. The known animal strains would thus be classified in the genus *Haemobartonella*.

Eperythrozoon infection in mice.—In 1928, Schilling (237) and Dinger (51) almost simultaneously discovered a new ring-like organism in the blood of splenectomized mice and concluded that it differed from the

bartonellae. These observations have been amply confirmed, and Schilling's name of *Eperythrozoon coccoides* has been adopted for the mouse strain on the basis of priority.

Occurrence.—The infection is apparently widely distributed geographically, and has been described in various strains and stocks of mice in Europe (52, 238, 32, 120, 167), in Africa (302), and in America (63, 77, 163). Both laboratory and wild mice may harbor the organisms. Not all stocks of mice carry the infection, however, since Marmorston (163) found that 5 of 8 inbred strains were free from the disease. The incidence of infection in carrier stocks is usually high, varying from 50 to 100 per cent.

The natural disease.—As in bartonellosis, severe spontaneous infection with *E. coccoides* does not occur. Splenectomy is usually required to permit detection of the organisms in the blood, although rare organisms may be seen in animals having latent infections (237, 32, 33, 167). Other insults to the host, such as x-ray irradiation or experimental lymphatic leukemia (163), may be followed by the appearance of eperythrozoa in the blood stream. The only significant pathological change in carrier mice is an increase in the weight of the spleen to approximately twice normal (163). Histological examination may reveal phagocytosis of infected erythrocytes, but does not show a concentration of organisms in the spleen (52, 63).

Following splenectomy, the organisms appear in the blood after an interval of from 1 to 19 days, but the usual period is 2 to 4 days (237, 51, 52, 32, 63, 120, 122, 167, 163, 331). During the next 5 days they increase in numbers rapidly, showing a definite preference for polychromatophilic erythrocytes. At the height of the infection, almost all the red cells may be involved, but certain cells show an extreme degree of parasitism, containing 20 to 40 or more organisms which entirely cover the surface or form cap-like colonies. Free organisms are present in the plasma. The organisms then rapidly diminish in number in the course of 2 or 3 days, but may persist in small although variable numbers up to 6 months. The great majority of mice have no clinical signs of disease. A few may show ruffled fur and slight weight loss at the height of the infection. The blood changes are slight—mild anemia, increase in reticulocytes and polychromatophilic cells, and inconstant leukocytosis. No significant pathological changes are found. One exception to the usual clinical course has been reported by Galli-Valerio (83). Nine months after splenectomy and the initial infection the mouse sickened, lost much of the hair about the head, and died. Autopsy revealed emaciation, viscid conjunctival secretion, pale and edematous muscles, large soft liver, and dull red kidneys. Large numbers of *Eperythrozoon coccoides*

were found in the red cells and free in the plasma. No other cause of death was determined, although it is doubtful if an adequate search for other infectious agents was made.

A strain of *Eperythrozoon*, termed *E. dispar* and differing from *E. coccoides* in morphology and pathogenicity, has been found in field mice (*Arvicola arvalis*) and dwarf mice (*Mus minutus*) by Bruynoghe and Vassiliadis (32, 33, 37), and in the vole by Tyzzer and Weinman (298). No clinical signs or special alterations of the blood were noted.

Several instances of mixed infection with bartonellae and eperythrozoa have been described (157, 158, 121, 163, 60). In view of the interference which Tyzzer (296) found in white mice between *E. coccoides* and the deer mouse strain of bartonella, it is interesting to note that Marmorston (163) encountered the same phenomenon in splenectomized mice spontaneously developing infection with natural strains of both organisms. In each of 4 animals showing mixed infections, the bartonellae became evident only after the eperythrozoa had disappeared. Moreover, when blood of these animals was injected into young splenectomized mice, only the *E. coccoides* developed.

Transmission of *E. coccoides* from infected to uninfected splenectomized mice by the mouse louse, *Polypax serrata*, appears to be a natural method of spread (62). Attempts to induce infection by other vectors, by contact, by a deficient diet, and by hereditary transmission have all been unsuccessful (52, 63, 167, 331).

Chemotherapy with arsenical compounds is effective in preventing or eliminating infection with *E. coccoides* (32, 33, 120, 122).

The experimental disease.—Infections with *E. coccoides* may be readily transmitted to splenectomized white mice free from the disease by subcutaneous or intraperitoneal inoculation of blood from an actively or latently infected mouse (237, 52, 32, 33, 331). The resulting disease is entirely similar to the natural infection. Injection of normal, disease-free mice produces a latent infection which may at any time be activated by splenectomy (331, 163). Reinoculation of chronically infected splenectomized mice is without effect (52, 331), but if the organisms are eradicated by arsenical therapy a second inoculation will reproduce the original infection with little or no evidence of immunity (32, 33). Attempts to infect by the oral route have been unsuccessful (331). Citrated blood retains its infectivity for 5 days but not for 10 days at 5°C. (331).

Inoculation of other species of animals with *E. coccoides* has given conflicting results. In normal rats a latent infection, becoming evident after

splenectomy, has been produced (63, 77), whereas in splenectomized rats immediate infection appeared (52, 32, 33, 63, 122). McCluskie and Niven (167), however, failed to confirm these results. Infection of splenectomized rabbits has been successful in some hands (35, 37), but not in others (120). Certain individual splenectomized voles (*Microtus pennsylvanicus*) have been found to be susceptible (296).

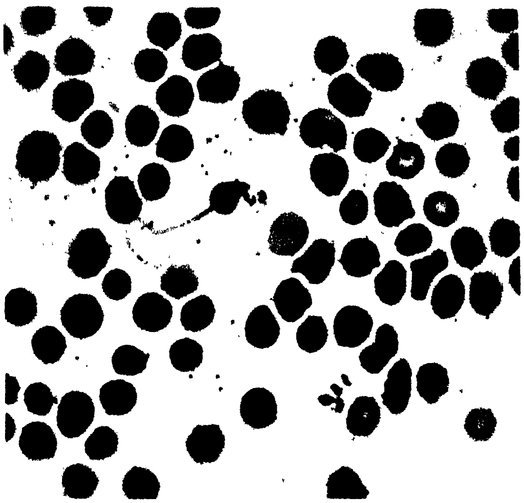


FIG. 165.—*Eperythrozoon coccoides.* Spontaneous infection in splenectomized laboratory mouse. Note organisms on the margins of the erythrocytes and free in the plasma. Giemsa. (×900.) (*Courtesy of Dr. D. Weinman.*)

The role of the spleen in this infection appears to be entirely similar to that in bartonellosis. No satisfactory attempts to demonstrate immunity have as yet been made.

Etiology.—*Eperythrozoon coccoides* characteristically appears in the blood of infected animals as a small gram-negative ring- or disk-shaped organism (Fig. 165), occurring either attached to the surface of erythrocytes or free in the plasma (237, 51, 52, 167, 63, 163, 331). It may be visualized by dark field technique or in dry blood smears stained with Wright's or Giemsa's stains. The rings are fairly uniform, approximately 1 μ in diameter, and have an unstained central zone with a bluish-red or violet periphery. Masses of cytoplasm at one or two points in the circumference may stain more intensely, giving the appearance of polar bodies. Some variation in shape occurs and racquet- or club-like forms may be seen. In addition, fine bacillary and tiny coccoid forms occur, quite distinct from the rod-like appearance of the rings which are viewed on edge. Organisms attacheP to

erythrocytes typically stain more intensely than those free in the plasma. Other stains, such as hemotoxylin, cresyl-blue, and azure II, stain them poorly if at all. No motility has been noted by darkfield examination.

E. dispar (32, 33, 37, 298), found in *Mus minutus*, *Arvicola arvalis*, and *Microtus pennsylvanicus*, is predominantly discoid in its morphology but is differentiated from *E. coccoides* chiefly by its animal pathogenicity. *E. coccoides* does not infect *Mus minutus* and *Arvicola arvalis*, whereas *E. dispar* fails to infect rats and white mice.

Attempts to cultivate the organism have in most instances been unsuccessful. Dinger (52) has reported cultivation on a coagulated egg medium in Locke's solution. Although no ring forms were seen in the culture, inoculation of a splenectomized mouse after three transfers produced the typical disease.

Grahamella infection in mice.—In 1905, Graham-Smith (91) described a new type of organism situated within the red blood cells of the mole. The organisms stained blue with Giemsa's dye, and appeared as long or short, curved, irregular rods, occasionally wedge- or club-like in shape. Dark chromatin masses sometimes occurred at one or both ends of the rods. The only pathological changes found were areas of focal necrosis in the livers of the most severely infected animals. This observation was soon confirmed by Thomson (272), who further noted that the bodies were non-acid-fast and gram-negative. He could not transmit them to the rabbit, guinea pig, rat, or mouse. Brumpt (31) in 1911 proposed the generic name of *Grahamella* for these organisms. Similar structures have subsequently been found in the erythrocytes of the common laboratory and wild mice and related species (212, 45, 21, 34, 36, 234). Classification of these organisms has not been settled (31, 96, 141, 36), and probably will not be established until cultivation has been achieved.

The incidence of the condition in mice may be high (34), but no adequate statistics are available. Infection does not result in clinically apparent disease or in recognizable pathological changes. Splenectomy is without pronounced effect (34). Examination of the blood of infected animals reveals that the organisms are generally restricted to the erythrocytes (45, 298) and may appear or disappear irregularly in a given animal (36). The percentage of affected cells is low, even in heavy infections (34, 2, 122), but the number of organisms in such cells is usually high, varying from about 5 to 20 or 30. The grahamellae differ from the bartonellae and eperythrozoa in being more or less uniformly spaced within the erythrocyte. They are considered to be non-pathogenic (2, 122), and attempts to transmit them to other animals have failed (141, 36), although final evaluation must await

further work. Chemotherapy with arsenical compounds has no apparent effect (34).

The mouse grahamellae, termed *Grahamella musculi* by Benoit-Bazille (21), somewhat resemble bacillary bodies in their morphology (45, 21, 34, 120, 122). They appear as rather coarse, irregular rods which may be club-shaped or beaded (Fig. 166). Variation in length (0.5 to 2 μ) is greater than that in width (0.2 to 0.3 μ). They are stained a more intense blue by Giemsa's method and are more azurophilic than the bartonellae and eperythrozoa. Other aniline dyes also stain them well. No adequate attempts to culture the organisms have as yet been reported.

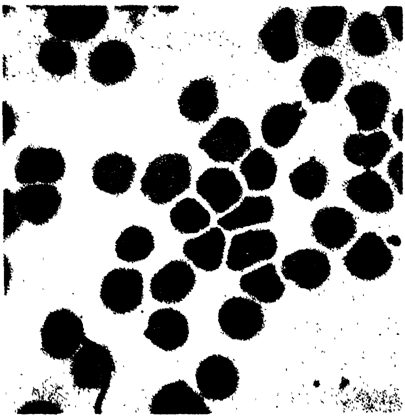


FIG. 166.—*Grahamella musculi.* Spontaneous infection in a laboratory mouse. Giemsa. (×900.) (*Courtesy of Dr. D. Weinman.*)

MISCELLANEOUS BACTERIAL INFECTIONS

Mice are highly susceptible to a considerable number of pathogenic bacteria which but rarely, if at all, cause spontaneous illness in these animals. Thus the anaerobic spore-forming bacilli (*Clostridia*) produce rapidly fatal diseases following administration of toxin or cultures. The organisms are commonly found in the feces of animals, yet natural infection has not been reported. Spontaneous tuberculosis caused by the avian tubercle bacillus has been found in mice (110, p. 633), infection apparently being acquired through the ingestion of avian feces. The enterococci and proteus organisms other than *Proteus morgani* (see p. 388) may infrequently produce disease in mice (94). Of more interest and importance, however, are two epidemics of tularemia in mice (200, 117). One occurred in Contra Costa County, California, among meadow mice (*Microtus californicus aestuarinus*); the other in the Kotelnikovo region of the Stalingrad district, Russia, among common mice (*Mus musculus*). In both instances the death rate was high and *Pasteurella tularemia* was isolated from afflicted animals. The disease was readily reproduced in mice experimentally (see also 78).

FUNGUS DISEASES

Infection of the skin with fungi, commonly called "favus" or "ringworm," is not uncommon in mice (110, p. 627; 299, 170, 249), and may even

spread epidemically (58, 197). Sporadic cases may be recognized by the denuded plaques usually present on the head or trunk. The skin in these areas is thickened; and disk-like, whitish-yellow crusts or scales cover the lesions. At the margins, the hair is of poor texture and easily pulled out. Diagnosis may be made by culture of the infected tissue on Sabauraud's agar and by microscopic examination of hairs or scales mounted on slides in 10 to 20 per cent sodium or potassium hydroxide solution. After 20 or 30 minutes mycelia and spores of the fungus may be seen both inside and outside the hairs. Various types of fungi have been reported: *Achorion quinckeanum, A. Schoenleinii, Trichophyton ectothrix megalosporium, and T. gypseum. Achorion quinckeanum* (*Sabouraudites quinckeanus, Microsporum quinckeanum*) is generally considered to be the common cause of favus of mice. There is considerable disagreement concerning classification of the fungi and identification of the various species is difficult. Details may be found elsewhere (215, 66, 340, 85). In general, spontaneous recovery occurs but treatment can be effected by the application of tincture of iodine, Whitfield's ointment, or a mercurial ointment. Care must be taken in handling infected animals, since the fungi readily infect man.

DuBois (58) has described an infection with *T. gypseum granulatum* involving 5 to 9 mice in a single cage. The first 3 animals affected showed an inflammatory type of cutaneous reaction with follicular suppuration. The lesions progressed slowly over the skin, producing complete loss of hair and toxic cachexia resulting in death. No visceral involvement was found at autopsy. The 2 other mice developed only focal areas of involvement without suppuration and recovery took place in 4 weeks. Microscopic examination and cultures of both types of lesions revealed the same organisms. Experimental inoculations produced only the attenuated type of infection.

Parish and Craddock (197) encountered an extensive epidemic of trichophytosis due to *T. gypseum asteroides* among a breeding stock of 2500 mice. The onset was sudden, 400 mice developing signs of the disease within a few days. Although the spread of the disease during the next 6 weeks was slow, recrudescence occurred and over 1000 mice of all ages were affected, necessitating destruction of the colony.

The lesions most frequently were situated on the neck, but were common on the back and rump. They consisted of bald patches with inflammatory thickening and scaliness of the skin. At the margins the hair had lost its luster, was brittle, easily detached, and in the worst cases the entire coat had

an "unhealthy, bristling" appearance. The tendency of the infection seemed to be towards recovery. Spread occurred by contact.

Examination of hairs from the periphery of the lesions after treatment with potassium hydroxide revealed chains of spores in the medulla of the hairs. Individual spores were oblong or square with rounded corners, and measured 3 to 6 μ in diameter. After about 3 days of cultivation on Sabauraud's maltose agar, tufts of growth appeared, enlarging to form white disks with a chalky, central opaque mass and a large powdery areola. After subculture, the margins presented uneven, ray-like prolongations and the reverse side of the colonies was brownish-red in color. The disease was reproduced experimentally by rubbing cultures into depilated and scarified skin. Further studies on transmission and immunity were terminated because of infection in the laboratory attendants. Ringworm of a different type was discovered in 4 of several hundred mice from a different stock, housed in another department of the laboratory.

Spirochetosis and Leptospirosis in Mice

Spiral organisms are not of significance as the etiological agents of fatal disease in mice. They are of importance, however, because mice may be carriers of the organisms which, in turn, may manifest themselves during the course of experimental or diagnostic procedures. Spirochetes have been found in spontaneous and transplanted tumors of mice (27, 294, 86, 41), and in the blood of animals inoculated with trypanosomes (334, 30, 99). The identity of these strains has not been established satisfactorily and various names have been applied to them: *Spirochaeta muris* or *Borrelia muris* (22), *S. microgyrata* var. Gaylordi, *S. laverani*, *S. naganophilia*, and others. Some of the organisms encountered probably were identical with *Spirillum minus;* others may have been saprophytic intestinal forms, or strains introduced by contamination of blood or tissue used for inoculation. Two forms, namely *Spirillum minus* and *Leptospira icterohemorrhagiae*, are worthy of further discussion because of their relationship to human disease.

Spirillum minus.—This organism, which was first found in a rat by Carter in 1887, has subsequently been shown to cause one type of rat-bite fever. It is identical with *Spirochaeta morsus muris*, and probably with *S. laverani* and *S. muris*. Varying figures have been given for the occurrence of the organism in laboratory (221) and wild mice. It has been found in the blood of one of two field mice (38), in the mammary glands of 31 of 33

lactating albino mice (149, 150), in the seminal vesicles* of 33 of 34 male mice, and in Bartholin (clitoral) glands* of 6 female mice (260), in the blood of 15 of 31 apparently normal white mice (131), in 6 of 8 white mice of one stock and 65 of 150 mice (two examinations) of a different stock (79). Other investigations give the incidence as 1 to 4 per cent (338, 241).

Mice infected spontaneously or experimentally usually remain healthy, showing no signs of illness and only slight splenic enlargement if killed. With repeated passage, however, the spirillum may become more virulent and produce death in approximately 14 days (221). Organisms appear in the blood 9 to 15 days after inoculation, gradually increase in number for 1 or 2 weeks, then slowly decline (241, 267, 222). At the height of the infection one or more spirilla may be found per microscopic field by dark field examination. Rarely, they may be demonstrable as long as 11 months after injection. The susceptibility of the mouse and the ease of demonstrating the organisms would make this animal an ideal one for diagnosis of the disease in other animals were it not for the above incidence of natural infection.

Transmission of infection in wild or stock animals presumably occurs by biting and contamination of food and water with urine (241). Hereditary transmission is both affirmed (11, 150) and denied (267). Infection of suckling mice by ingestion of organisms in the milk (149, 150) and from mouse to mouse by sexual contact (260) has been proposed.

Spirillum minus is a rapidly motile, rigid organism, having from two to six regular spirals. The ends taper and are provided with one or more flagella. It is perhaps best observed by dark field examination, but it can be stained by aniline dyes or by silver impregnation methods. The organism probably should be classified as a bacterium in the same family as the vibrio group, although common usage includes it with the *Spirachaeta*.

Various strains isolated from mice, rats, and cases of human infection have been studied rather extensively in an attempt to differentiate them (338, 223, 240, 241). Some differences in virulence and serological reac-

* The identification of the spiral organisms found by Stroesco (260) in the seminal vesicles and Bartholin's glands as *Spirillum minus* was based on morphological appearance in stained sections of tissue. Dubois (59) has shown that this organism is more like a spirochete in morphology and motility and further differs from *S. minus* in pathogenicity and resistance to chemotherapeutic agents. Both Dubois and Mackenzie (160) consider it to be a new, non-pathogenic species which must be differentiated from *S. minus*.

tions have been found by various investigators, but the detailed work of Schockaert (240, 241) indicates that virulence varies regardless of source and that the human, rat and mouse strains constitute a single species. Human cases resulting from the bite of a mouse (111, 115, 217), or from inoculation of a mouse strain of the spirillum (131, 240), are indistinguishable clinically from those due to human or rat strains.

Recovery from infection and disappearance of the organisms in mice probably is due to an immunological mechanism. Although lytic antibodies have never been satisfactorily demonstrated in mice, active immunization to homologous and heterologous strains does occur. Arsenic preparations, such as arsphenamine or neoarsphenamine, are effective in treating human infection and might be tried if it were desirable to rid infected mice of the organisms.

Leptospira icterohemorrhagiae.*—Following the identification of *Leptospira icterohemorrhagiae* as the causative agent of infectious jaundice (Weil's disease) and its demonstration in rats, Miyajima [quoted by Ido, et al. (108)] found the organism on several occasions in the kidneys of the field mouse, *Microtus montebelloi*. The leptospira was subsequently found in 1 of 6 field mice (108), and in 1 of 2 field mice but not in 2 house mice from the Edinburgh area of Scotland (38). Packchanian (193) has recently reported the occurrence of *Leptospira icterohemorrhagiae* in laboratory white mice (*Mus musculus*) and the susceptibility of certain species of American deer mice (*Peromyscus*) to experimental infection. White mice ordinarily show no signs of infection. Inoculation of infected mouse blood or tissues into guinea pigs or American deer mice, however, results in fever, jaundice, hemorrhages, and death. The organisms are demonstrable in the blood and urine.

Morphologically, *Leptospira icterohemorrhagiae* is a delicate organism having closely wound, rigid spirals and secondary wavy curves. It varies from 0.1 to 0.2 μ in width and from 6 to 12 μ in length, occasional specimens being as long as 25 μ. The organism is flexible and one or both ends may be curved or hooked, giving an S or C shape. During movement, the hooked ends whirl around rapidly. Darkfield examination and silver impregnation are best for demonstration of the organism. Bile salts (10 per cent) but not saponin dissolve it. Cultivation may be effected in dilute serum media at 25°C.

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* Sellards (248) has recently proposed *Leptospira interrogans* as the correct name for this organism.

lactating albino mice (149, 150), in the seminal vesicles* of 33 of 34 male mice, and in Bartholin (clitoral) glands* of 6 female mice (260), in the blood of 15 of 31 apparently normal white mice (131), in 6 of 8 white mice of one stock and 65 of 150 mice (two examinations) of a different stock (79). Other investigations give the incidence as 1 to 4 per cent (338, 241).

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Various strains isolated from mice, rats, and cases of human infection have been studied rather extensively in an attempt to differentiate them (338, 223, 240, 241). Some differences in virulence and serological reac-

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* The identification of the spiral organisms found by Stroesco (260) in the seminal vesicles and Bartholin's glands as *Spirillum minus* was based on morphological appearance in stained sections of tissue. Dubois (59) has shown that this organism is more like a spirochete in morphology and motility and further differs from *S. minus* in pathogenicity and resistance to chemotherapeutic agents. Both Dubois and Mackenzie (160) consider it to be a new, non-pathogenic species which must be differentiated from *S. minus*.

tions have been found by various investigators, but the detailed work of Schockaert (240, 241) indicates that virulence varies regardless of source and that the human, rat and mouse strains constitute a single species. Human cases resulting from the bite of a mouse (111, 115, 217), or from inoculation of a mouse strain of the spirillum (131, 240), are indistinguishable clinically from those due to human or rat strains.

Recovery from infection and disappearance of the organisms in mice probably is due to an immunological mechanism. Although lytic antibodies have never been satisfactorily demonstrated in mice, active immunization to homologous and heterologous strains does occur. Arsenic preparations, such as arsphenamine or neoarsphenamine, are effective in treating human infection and might be tried if it were desirable to rid infected mice of the organisms.

Leptospira icterohemorrhagiae.*—Following the identification of *Leptospira icterohemorrhagiae* as the causative agent of infectious jaundice (Weil's disease) and its demonstration in rats, Miyajima [quoted by Ido, et al. (108)] found the organism on several occasions in the kidneys of the field mouse, *Microtus montebelloi*. The leptospira was subsequently found in 1 of 6 field mice (108), and in 1 of 2 field mice but not in 2 house mice from the Edinburgh area of Scotland (38). Packchanian (193) has recently reported the occurrence of *Leptospira icterohemorrhagiae* in laboratory white mice (*Mus musculus*) and the susceptibility of certain species of American deer mice (*Peromyscus*) to experimental infection. White mice ordinarily show no signs of infection. Inoculation of infected mouse blood or tissues into guinea pigs or American deer mice, however, results in fever, jaundice, hemorrhages, and death. The organisms are demonstrable in the blood and urine.

Morphologically, *Leptospira icterohemorrhagiae* is a delicate organism having closely wound, rigid spirals and secondary wavy curves. It varies from 0.1 to 0.2 μ in width and from 6 to 12 μ in length, occasional specimens being as long as 25 μ. The organism is flexible and one or both ends may be curved or hooked, giving an S or C shape. During movement, the hooked ends whirl around rapidly. Darkfield examination and silver impregnation are best for demonstration of the organism. Bile salts (10 per cent) but not saponin dissolve it. Cultivation may be effected in dilute serum media at 25°C.

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* Sellards (248) has recently proposed *Leptospira interrogans* as the correct name for this organism.

A somewhat similar organism, termed *Leptospira aquicole pseudoictero-genica*, has been reported in the kidneys and urine of stock mice (23). The strain apparently is identical with one found in water.

Virus Diseases of Mice

Filterable viruses have been known to produce a variety of diseases in plants and animals since 1892. Only in the past 10 years, however, have spontaneous diseases due to the viruses been recognized in mice. Some of these diseases may spread within a mouse colony, producing highly fatal results. Others may produce no visible signs of infection but are equally important, since they may evidence themselves in the course of experimental procedures.

The virus diseases to be discussed are infectious ectromelia, lymphocytic choriomeningitis, encephalomyelitis of mice (Theiler), virus pneumonia, and salivary gland disease. The etiological agents are acceptable as viruses since they fulfill one or more of the following criteria: transmissibility, filterability, failure to grow on non-viable culture media, absence of cultivable bacteria, presence of inclusion bodies in the cells of the host, and production of immunity.

Infectious ectromelia.—In 1930 Marchal (162) described a new virus disease of mice. It occurred principally in young mice and was noted most frequently when the animals were separated from their mothers and placed together in groups of 50. Subsequently, the spontaneous disease has been found in England (169) and on the Continent (24, 242, 102), but has not yet been reported from this country. It occurs in laboratory mice of different stocks and has been found in wild mice caught in the laboratory. McGaughey and Whitehead (169) found the disease to be so widespread in England that difficulty was experienced in obtaining healthy mice. Some animals apparently harbor the virus and develop the disease only when subject to experimental inoculation (169, 102).

The natural disease.—Clinically, the disease occurs in two forms. The acute or abdominal type usually appears first in a stock of infected mice and is evidenced by loss of normal activity and ruffled, lustreless coats without other significant signs. No skin lesions are present. Death may occur after an illness as short as 4 hours, and the fatality rate may reach 80 to 90 per cent in certain lots of mice. Recognition of this form may be difficult unless careful autopsies are performed.

The chronic or cutaneous form of the disease appears later in animals surviving the acute type or in those previously uninfected. Here skin

manifestations form a prominent part of the clinical picture and are almost pathognomonic. Enlargement is noted in one foot—usually a hind foot—which presents a swollen translucent appearance due to edema of the subcutaneous tissues. As the edema increases, exudation of serous fluid occurs through the skin and crusting takes place over superficial ulcers. Vesicles may also form. The diseased skin is usually sharply demarcated from healthy tissue by a line of constriction, and gangrene of a toe or the foot may follow, with ultimate separation of the foot at this line. Recovery ordinarily occurs in these cases, and the animals are then immune to subsequent expo-



FIG. 167.—Infectious ectromelia: left, advanced lesions of foot and leg; right, normal mouse. (*From McGaughey and Whitehead, J. Path. and Bact.*)

sure or inoculation. Should the disease spread to involve any or all of the other feet, the tail, or the skin around the mouth or over the body, death invariably results (Fig. 167).

Pathology of the natural disease.—Mice dying acutely show an increase in peritoneal, pleural, and pericardial fluid which at times may be abundant. Serous membranes, especially those of the intestines, are markedly congested. The liver is usually pale and anemic or greyish-brown in appearance and soft and necrotic in consistency. The spleen is ordinarily normal in size or but slightly enlarged, and may be either studded with yellowish-grey areas of necrosis or present an appearance of massive necrosis. Congestion is present in the lymphatic glands, lungs, and sometimes the adrenal cortex. No organisms can be found by direct smear or by culture of the exudate or organs. Aside from generalized congestion and occasional small hemor-

rhages, the microscopic picture is one of diffuse necrosis in the liver and spleen. The characteristic feature, however, is the presence of intracytoplasmic eosinophilic inclusion bodies in the epithelial cells of the intestine and the acinar cells of the pancreas (Fig. 168). These inclusions occur singly or in small groups, and vary in size up to 7 μ. Only one observer (242) has noted them in the liver.

The lesions in the chronic or cutaneous type of infection are more extensive. Those in the skin consist of crusted, superficial ulcers surrounded by indurated, edematous tissue. Ascites, hydrothorax, and congestion are present. The liver is a mottled red color and contains numerous greyish-white areas of necrosis. The spleen is enlarged and red with similar necrotic areas. Over the peritoneum and serosal surface of the viscera may be found whitish areas suggestive of fat necrosis. The kidneys are usually normal but may be enlarged and pale, resembling those of the second stage of nephritis. Microscopic examination confirms the gross evidence of an extensive necrotizing process which involves the skin, liver, spleen, peritoneum, and other tissues. Perivascular cuffing and fatty degeneration are pronounced in the liver. Affected kidneys show groups of endothelial-like cells in the cortex, small hemorrhages, and fatty degeneration of the convoluted tubules. Intracytoplasmic inclusion bodies are most numerous in the epithelial cells of the skin, where they may be as large as 13 μ in diameter (Fig. 169). They are also found in connective tissue cells and endothelial cells of vessels in the subcutaneous tissue, epithelial cells of the intestine, acinar cells of the pancreas, secretory cells of the salivary glands, and epithelial cells of the tongue and lips. The larger inclusions are round or oval in shape, stain evenly with acid dyes, and as they enlarge cause degeneration and finally disappearance of the nucleus. For demonstration of the bodies, the tissue may be fixed in a saturated solution of bichloride of mercury containing 5 per cent glacial acetic acid, and stained with the ordinary hematoxylin-eosin stain. Mann's methyl blue eosin or acid fuchsin and Weigert hema-



FIG. 168.—Infectious ectromelia: inclusion bodies (black) in cells of the pancreas. Mann's stain. (×1000.) (*From McGaughey and Whitehead.*)

toxylin may be used. After chromic acid fixation, methylene blue gives a characteristic picture.

The experimental disease.—The disease can be transmitted to normal mice by inoculation of edema fluid, blood, or various tissue emulsions from infected animals. Blood plasma is infective from 1 to 5 days after the appearance of the lesions, the liver and spleen after 3 days. Intradermal inoculation in the foot pad usually reproduces the cutaneous type of disease. Intraperitoneal or intravenous injection produces the acute form, with

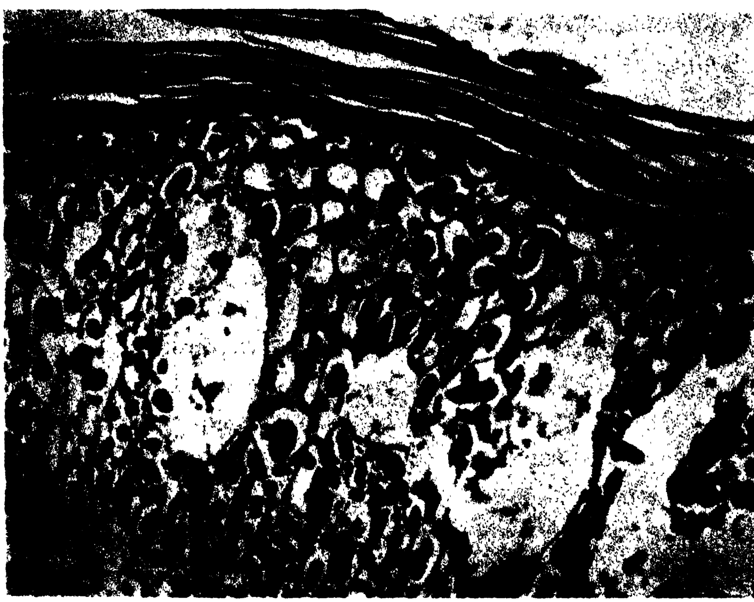


FIG. 169.—Infectious ectromelia: section of skin showing edema, necrosis, and inclusion bodies in the epithelial cells. Hematoxylin-eosin. (×360.) (*From Birsch-Hirschfeld.*)

death in 4 to 6 days. A disseminated pneumonia follows inhalation of the virus. Transmission of infection also occurs by permitting contact of normal and infected mice under ordinary conditions, which has permitted study of experimental epidemics of the disease (92, 119).

Properties of the virus.—The virus responsible for this disease may be isolated from the liver, spleen, central nervous system, lymph nodes, lungs, peritoneal and edema fluids, and blood. It is filterable through diatomaceous or porcelain filters (Chamberland L2, Mandler, Berkefeld N) and has an estimated size of 100 to 150 mμ (18). It is resistant to drying in a desiccator, may be preserved for months in 50 per cent glycerin at 0°C., but is inacti-

vated by 0.01 per cent formalin in 48 hours and by a temperature of 55°C. in 30 minutes. Studies of the inclusions (18, 98, 144) have revealed the presence of elementary bodies entirely similar to the Paschen bodies of vaccinia and the Borrel bodies of fowlpox. The inclusions are resistant to digestion with trypsin (24). Propagation of the virus has been successful only in tissue culture and on the chorio-allantoic membrane of the egg (56, 57, 199, 40).

The virus is strikingly species-specific. Only mice show signs of the disease, although inapparent infection has been demonstrated in the rat (39). One attack of the disease confers a solid immunity upon the survivors, in whose serum neutralizing antibodies are demonstrable. Attempts to produce immunity by the use of inactivated virus have thus far been unsuccessful (24), and prevention of the disease can be accomplished only by general measures.

An epidemic disease, somewhat similar to ectromelia, has been reported by Thompson (273, 275). Intracytoplasmic and intranuclear inclusion bodies were found in the parenchymatous cells of the liver, but were absent from epithelial tissues. Further comparison of the two diseases was not reported.

Lymphocytic choriomeningitis.—The virus of lymphocytic choriomeningitis was first obtained from mice by Traub (291, 292) in 1935. It was encountered during his work with the viruses of equine encephalomyelitis and hog cholera and was found to be distinct immunologically and pathologically from both of them. In spite of the fact that he at first had recognized no disease in his mouse colony, it seemed likely that the mouse was the natural host of the virus. Accordingly, he inoculated a group of 60 5-week-old mice with sterile bouillon by the intracerebral route. Nine of these animals developed symptoms in from 3 to 13 days, and 4 died. No bacteria were obtained by culture. Inoculation of suspensions of brain into guinea pigs reproduced the disease. From later studies, he estimated that about 50 per cent of the mice were infected with this agent, which was identified as the virus of lymphocytic choriomeningitis (292, 219, 220).

The original isolation of the virus was reported in 1934 by Armstrong and Lillie (14), who accidentally encountered it in monkeys during the course of their studies on the epidemic of encephalitis in St. Louis. The origin of this strain was not definitely determined. Subsequently, the virus has been isolated from cases of meningitis in man (219, 246, 69, 138, 15), and from laboratory and house mice (69, 146, 138, 15). The latter observation is of particular interest since the virus was found in grey mice (*Mus musculus*) trapped in two houses in which human cases occurred (15). Accidental

infection of a laboratory worker (147) and experimental reproduction of the disease in man by inoculation with the murine virus (145), together with the above reports, suggest that mice constitute a natural reservoir of the disease.

The natural disease.—In most instances in which the virus of lymphocytic choriomeningitis has been found in mice the infected animals have appeared to be entirely normal and healthy and the disease has existed as a latent infection. In the colony observed by Traub (292), however, signs of infection were noted. He describes the animals as follows: " . . . a number of 2 to 6 week old mice were emaciated and drowsy. Their fur was ruffled and they were often seen sitting in corners of the cage by themselves. Their movements were slow and stiff, and their legs appeared long in proportion to their thin bodies." Other signs of infection were conjunctivitis, photophobia, and a slow rate of growth. No signs of involvement of the central nervous system were noted. Approximately one-half of the mice in the colony were infected, although the morbidity was less than 20 per cent, and the mortality less than 2 per cent of the number of infected animals. The majority recovered completely in 3 weeks. The active agent was isolated from the blood and brain of apparently normal mice, of those showing only conjunctivitis and photophobia, and of those obviously ill or found dead.

No gross lesions were found at autopsy of these animals. Microscopic examination of the liver revealed slight perivascular round cell infiltration, scattered lymphocytic infiltration in the interstitial tissue, and patchy reticulo-endothelial hyperplasia. Slight peribronchial and perivascular infiltration with round cells and slight thickening of the alveolar walls were found in the lungs of 2 of 12 mice. Only 1 mouse showed a slight meningeal reaction consisting of lymphocytic cells.

Transmission of the natural disease presumably occurs by contact, since normal mice may be infected by placing them in a cage with diseased animals. Although the route of infection is not definitely known, it has been demonstrated that the virus is often present in the urine and nasal secretions of diseased mice (292). The agent has also been found in embryo, new born, and suckling mice (138).

The experimental disease.—Laboratory and wild mice are susceptible to virus introduced by almost any route of inoculation, but only by intracerebral injection is a definite clinical picture produced. Following such administration, an incubation period of 5 to 7 days elapses before the mice appear ill. They then show signs of malaise—lassitude, ruffled fur, hunched back, and partially closed eyes. Death may occur suddenly without other

signs of infection, but more commonly the animals become hyperactive so that even a slight stimulus will cause them to leap into the air or will induce a convulsion. If the mouse is lifted by the tail, a convulsion frequently follows, characterized by rapid clonic movements of the fore legs, terminating in a sustained tonic extensor spasm of the hind limbs, and lasting from one to several minutes. Convulsive attacks also occur spontaneously. Death may result in the first or subsequent attacks. If the animals survive for 3 or 4 days after the onset of signs of the disease, complete recovery without residual paralysis usually occurs. Blood counts are within normal limits (292). This same clinical course may be seen in naturally infected mice injected intracerebrally with sterile starch emulsion or bouillon.

Intranasal and subcutaneous inoculations produce no signs of the disease, but the virus may be demonstrated in the blood and the animals acquire an immunity to subsequent intracerebral inoculation. Mice inoculated intraperitoneally or intravenously may show labored respiration 5 to 10 days later for a period of a week or more. Convulsions do not occur. A few of the mice die, but the majority recover and are resistant to a second inoculation. The virus may persist for weeks or months in mice recovering from experimental infection and has been demonstrated in the brain, blood, liver, spleen, kidneys, lungs, adrenal, nasal passages, and urine. No neutralizing antibodies, however, have been observed in the blood of recovered mice (293).

The pathological picture varies with the route of inoculation. Mesodermal tissues are primarily involved with the production of a hyperplastic reaction. Following intracerebral inoculation, congestion is apparent grossly in the surface vessels of the brain, in the liver, and in the spleen, which may be slightly enlarged. Microscopically, there is infiltration of the meninges of the brain and spinal cord, the cellular exudate being composed chiefly of lymphocytes, and to a less extent, of mononuclear and polymorphonuclear cells. Infiltration is most marked at the base of the brain (Fig. 170), but the choroid plexuses and the ependyma are quite constantly involved. Perivascular round cell infiltration is present if the animals survive for 2 or 3 weeks. Involvement of the nervous tissue proper is minimal, and no inclusion bodies are found. Changes in the other organs are minor; irregular hyperplasia of reticulo-endothelial (Kupffer) cells and slight lymphocytic infiltration in the liver, and small areas of interstitial bronchopneumonia in the lungs are the chief findings.

Mice developing signs of infection after intraperitoneal or intravenous inoculation show visceral lesions but infrequently there is evidence of even

slight meningitis. The significant findings are enlarged spleen, a pale or nutmeg liver, serous pleuritis and peritonitis, lungs which may appear normal or contain small areas of consolidation, and occasionally pale and slightly swollen kidneys. Microscopically, there is generalized proliferation of the reticulo-endothelial cells, interstitial and perivascular round cell infiltration, and interstitial bronchopneumonia. Rarely is there necrosis of parenchymal cells. Blood counts in mice injected intravenously may show



FIG. 170.—Lymphocytic choriomeningitis. Marked meningitis at the base of the brain of a mouse inoculated intracerebrally with the virus. Eosin and methylene blue. (×130.) (*From Traub.*)

a leukocytosis up to 55,000 per c. mm. with a relative and absolute lymphocytosis and monocytosis.

Cultures of various tissues from infected animals reveal no bacteria of possible etiological significance, and the disease may be reproduced by the inoculation of filtrates of tissue emulsions after passage through Berkefeld or Chamberland candles. Guinea pigs are particularly suitable for inoculation since they are highly susceptible, do not themselves carry the virus, and react with a characteristic clinico-pathological picture due to a slowly progressing pneumonia (292). Intracerebral, subcutaneous, and intranasal routes of inoculation may be employed.

Properties of the virus.—As already pointed out, the virus of lymphocytic choriomeningitis is mesodermatropic in nature and is widely distrib-

uted in the tissues of infected animals. Different strains vary in the degree of their virulence, but in general the virus is pathogenic for mice, rats, guinea pigs, monkeys, and man. The serum of certain convalescent or recovered animals contains complement-fixing and protective antibodies, as does that of rabbits inoculated with virus suspensions (106, 147, 251, 252, 253).

The virus withstands freezing and drying (252), and 50 per cent glycerin for at least 1 month (291), but rapidly decreases in infectivity when suspended in physiological saline solutions at room temperature unless protected by the addition of 2 per cent normal inactivated serum (252). In size the virus particles are not more than 100 to 150 mμ in diameter, as determined by filtration through graded collodion membranes (220). From suspensions of infected tissue, a soluble antigen—apparently protein in nature—has been obtained (252, 253). It is capable of fixing complement and of precipitating when mixed with immune serum. The antibodies which react with this antigen are apparently distinct from those responsible for neutralization of the virus in protection tests. Immunological reactions with both tissue emulsions and soluble antigen are entirely specific, and no qualitative differences have been found between various strains of the virus.

Diagnosis of the disease.—Since the disease may be present in a mouse colony as a subclinical, latent infection, its existence may not be suspected. Recognition of the infection may be accomplished by several methods. (*a*) The virus may be isolated by intracerebral inoculation of an "indicator host" (7)—that is, a guinea pig or mouse known to be free from the infection—with blood or emulsion of brain, spleen, or kidney. (*b*) Demonstration of immunity in a certain number of stock mice is an indication of previous infection. Traub, for example, found that the morbidity rate following intracerebral inoculation of the virus into mice from the infected stock was about 60 per cent and the mortality rate about 40 per cent. (*c*) Intracerebral injection of a sterile, non-infectious agent, such as starch emulsion or bouillon, may be a sufficient stimulus in some of the animals to cause a flare-up of the inactive infection, resulting in a clinical picture similar to that seen in normal mice inoculated intracerebrally with the virus (292, 69, 146).

Final diagnosis of the disease is made on the basis of the clinical course in mice or guinea pigs, the pathological findings, and immunological identification of the virus by complement fixation or protection tests, or by inoculation of recovered animals with a known strain of the virus. Immunological methods, for example, afford a clear distinction between the viruses of lymphocytic choriomeningitis and acute meningo-pneumonitis (81), although the clinico-pathological features following intracerebral injection are similar.

No specific measures are as yet available for effective immunization of mice or for prevention of the disease. General preventive measures should be taken to protect a disease-free colony or to stop spread of the infection.

Encephalomyelitis of mice (Theiler).—Spontaneous encephalomyelitis of mice is a virus disease which rarely produces clinical signs under natural conditions. The active agent, however, is widespread in distribution. It may be obtained with great regularity from normal mice of certain age groups, or may be encountered in animals inoculated with other agents. Since Theiler (268) first described the disease and demonstrated its etiology the virus has been found in several strains of mice in the United States (269, 231, 270), as well as in Germany (88), Japan, (109), and Palestine (189).

Occurrence.—The incidence of the natural disease is difficult to determine, but is probably very low. Various figures have been given: 1 in about 2000 Swiss mice purchased from various dealers (269); 1 or 2 per 1000 mice of the Rockefeller strain (231) although no cases were found among a series of 5000 animals observed later (189). The low incidence does not indicate lack of contact with the infective agent, however, since the virus has been demonstrated in the intestinal contents of almost all (66 to 100 per cent) mice between the ages of 1 and 2 months (187, 271, 188, 189).

The natural disease.—Spontaneous illness in mice (268, 269) may be recognized by the development of flaccid paralysis of the hind legs without other apparent signs. No reports of the course and mortality are available. Pathological examination of the central nervous system reveals scattered necrosis of ganglion cells and perivascular infiltration, most marked in the spinal cord but also present in the brain. The disease seems to become evident chiefly in young mice—approximately 6 to 7 weeks of age—some of which are apparently highly susceptible to invasion of the central nervous system. There is no evident reason why certain animals should be afflicted while the great majority escape, yet practically all at this age are carriers of the virus. In paralyzed animals, the virus is present in the spinal cord in highest concentration and in the brain. It has not been demonstrated in the blood.

The virus is regularly found in the contents and walls of the gastrointestinal tract, in the mesenteric glands, and in the feces, but not in the central nervous system, salivary glands, or other organs of normal mice between the ages of 4 and 8 weeks. It is absent or irregularly present in animals younger than 20 days or older than 6 months. Excretion of the virus may persist up to 53 days after the first isolation (271).

Intracerebral injection of mice with other agents (109, 270) has resulted in the isolation of the most virulent strains of the virus yet obtained. No relationship could be established between the strain of murine encephalomyelitis isolated and the agents injected (the viruses of yellow fever and human encephalitis), so that exacerbation of a latent infection seems to be the most likely explanation. The signs of infection were entirely similar to those resulting from experimental intracerebral inoculation of known strains of the virus.

The experimental disease.—The production of clinical disease by experimental inoculation of the virus depends on the virulence of the strain of virus, the route of inoculation, and the age of the mice. With strains of relatively low virulence—those obtained from intestinal contents of normal mice or the central nervous system of naturally infected mice—intracerebral injection of young animals gives a high morbidity and mortality, whereas by intranasal inoculation only a low incidence of paralysis occurs. Other routes are ineffective (268, 269, 270, 88, 189). With strains of higher virulence (109, 270), however, signs of involvement of the central nervous system occur following intracerebral, intranasal, and intraperitoneal injection with greater regularity, and occasionally following subcutaneous inoculation. The influence of age is shown by the fact that the morbidity and mortality rates are lower and the incubation time longer in mice over 12 weeks of age (270, 84, 109).

After intracerebral injection (268, 269, 88, 109, 270), a period of 5 to 30 days may elapse before the appearance of signs of the disease, but the average time is 10 to 14 days. The first sign is a weakness of one limb, rapidly followed by flaccid paralysis of that member. The paralysis may spread to involve all four legs, but usually the hind limbs are more markedly affected so that locomotion is possible only by use of the fore legs. Atrophy, emaciation, and contractures of the involved members occur. Incontinence of urine may be observed in severely afflicted animals. In spite of the above evidence of damage to the nervous system, the mice do not appear acutely ill during the first stages of the disease. Finally, however, the fur becomes ruffled, respiration labored, and the animal succumbs. Mice 4 weeks of age or younger, however, may die without showing signs of infection. If recovery occurs, the extent of the involvement diminishes, but residual paralysis of the hind limbs is almost constantly present. Such recovered animals may harbor the virus in the spinal cord for more than a year (269). The duration of the disease from the first appearance of clinical signs to death or recovery varies between 2 and 10 days. Following other routes of

inoculation the clinical picture, if apparent at all, is essentially that described above.

No significant gross changes are found at autopsy (268, 269, 88, 109). Microscopic lesions occur primarily in the spinal cord and are characterized by perivascular round cell infiltration, acute neuronal necrosis particularly of the anterior horn cells, neuronophagia, and gliosis. Ganglion cells of the posterior root are not involved. The brain shows perivascular cuffing to a lesser extent and degeneration of occasional isolated neurons. A decrease in the number of anterior horn cells is found in the cords of recovered mice with residual paralysis. No inclusion bodies have been demonstrated. The virus may most easily be demonstrated in the spinal cord and brain.

The clinical picture produced by the two more virulent strains of virus isolated by Theiler and Gard (270) differs considerably from that described above. The incubation period following intracerebral inoculation is much shorter (2 to 6 days), the course more rapid (24 to 48 hours), the mortality greater, and the titer of virus in the infected brains higher. With one strain (FA) the signs of infection following intranasal or intracerebral injection resemble those of an encephalitis more than a myelitis, e.g., an appearance of being sick, hyperexcitability, ruffled fur, twitching, and tonic convulsions sometimes terminating in death. Weakness of one of the legs may occur but paralysis is rare. The histopathological appearance is that of a marked encephalitis with a minimal meningeal reaction. Following intraperitoneal inoculation, however, flaccid paralysis is usually the predominant sign. With the other strain (GD VII), hyperirritability may be the first sign of infection, but the mice appear well and the signs are referable to lesions of the cord, i.e., flaccid paralyses. The same picture results from intracerebral, intranasal, or intraperitoneal injection.

Properties of the virus.—Although the several strains of this virus vary in virulence or invasiveness, they are much alike, if not identical, in their other properties (268, 269, 270, 88, 109). The average particle diameter as determined by filtration through graded collodion membranes is 9 to 13 mμ, closely approximating that of the viruses of human poliomyelitis and of foot-and-mouth disease of cattle. Filtration through all grades of Berkefeld filters is accomplished with ease. The virus may be preserved in 50 per cent glycerin at 2° to 4°C. for more than 150 days and is most stable at pH 8.0 or pH 3.3. It withstands the action of ether and precipitation by ammonium sulphate, but is destroyed or inactivated by a temperature of 50°C. with rapidity, by 20 per cent ethyl alcohol in 45 minutes in the icebox, by 1 per

cent hydrogen peroxide in 2 hours at 37°C., and by desiccation in the frozen state at −16°C.

The virus is limited in its host pathogenicity. Mice are susceptible, but guinea pigs, rabbits, and *rhesus* monkeys are resistant. Theiler and Gard (270) have recently reported that one of their strains of encephalomyelitis virus is pathogenic for the cotton rat. This work is of considerable interest, since the cotton rat has been reported by Armstrong (12, 13) to be susceptible to the Lansing strain of human poliomyelitis virus, and the virus recovered from the inoculated animals was then found to be pathogenic for mice by the intracerebral route. An immunological relationship apparently exists between these two viruses, since Theiler and Gard (270) found " . . . that mice which had been infected with the virus of mouse encephalomyelitis were resistant to a subsequent intracerebral inoculation of Armstrong's Lansing strain of human poliomyelitis virus . . . " Jungeblut and Sanders (114) have also isolated a virus from a cotton rat injected with the SK (New Haven) strain of human poliomyelitis virus. The animal died a week after inoculation without presenting signs of disease, but subsequent passage of the agent to cotton rats resulted in flaccid paralysis of the hind legs and death. Inoculation of mice produced an illness clinically like mouse encephalomyelitis. Mice from a colony immune to the spontaneous murine encephalomyelitic agent, however, were susceptible to infection with their virus. All later attempts to produce infection in rats or mice with the original material were unsuccessful. Further investigation is necessary to clarify the relationship between the above strains of virus.

Immunologically, the murine strains of this virus thus far isolated are antigenically related (270). Recovered but paralyzed animals are resistant to a second inoculation regardless of the route used to infect them. Mice infected intracerebrally with a relatively avirulent strain of virus are immune to subsequent inoculation with a highly virulent strain (GD VII). Although the interference phenomenon might be responsible for erroneous conclusions in experiments of this type, the high degree of resistance to a second inoculation is more suggestive of true active immunity. The two more virulent strains (FA and GD VII) are not immunologically identical, however, since a greater resistance is produced by immunization with the homologous than with the heterologous strain. The greater resistance of older mice is probably due to previous contact with the virus (271, 189), but the same phenomenon is seen with other infectious agents and may be the resultant of anatomical and physiological (228), as well as immunological factors.

Neutralization of the virus by the serum of convalescent mice has not been satisfactorily demonstrated by the methods so far employed. The results suggest that relative protection can be conferred, but the degree of protection is insufficient to permit an immunological comparison of the various strains of virus by this method. The murine virus is not neutralized by antiserum for the virus of human poliomyelitis.

Differential diagnosis.—The clinical course of this disease following intracerebral inoculation is sufficiently distinctive to differentiate it from other encephalitis agents such as lymphocytic choriomeningitis, equine encephalomyelitis, etc., whereas the diameter of the virus, pathological findings, and host specificity distinguish it from acute meningo-encephalitis (81).

Final diagnosis is made on the basis of size, host range, pathology, and cross-protection. Parasitic meningo-encephalitis may be distinguished pathologically, and bacterial infections by cultural methods.

Epidemiology.—Considerable interest is centered in epidemiological studies (271, 189) of this disease because of its similarity to human poliomyelitis. The significant features thus far demonstrated are the widespread distribution of the virus as evidenced by its almost constant presence in the intestines of young mice, the low incidence of spontaneous disease, the prolonged period of excretion in the feces, and the gradual development of resistance with increasing age. The exact route of natural infection is not known, but in all probability is either nasal or oral since fecal excretion must keep the environment almost constantly infected. That such excretion by an infected mouse is not dependent on continuous infection, however, is shown by isolation experiments in which the opportunity for self-infection was minimal. Under such conditions, the intestinal wall is apparently the site of elaboration of the virus, and invasion of the mesenteric glands may occur secondarily. Whether the intestinal tract is the focus for distribution of the virus when first introduced, or is but secondarily infected, has not been determined. Theiler and Gard (271) have suggested that the development of antibodies due to infection of the intestinal tract may be responsible for the increasing resistance with age. The failure of an individual animal to form antibodies might then allow invasion of the central nervous system and the production of clinical disease. Experiments with a disease-free stock of mice would be of value in elucidating further the epidemiology and nature of this disease, which, though unimportant as regards mortality, is of considerable importance to an investigator employing mice in the study of viruses.

Virus pneumonia in mice.—Mice are being widely used for the investigation of certain human respiratory infections, such as influenza, because these animals respond to intranasal administration of the causative viruses with the production of pneumonic consolidation. In the isolation of the virus from nasopharyngeal washings from the patient, however, it is often necessary to make several "blind" passages of lung tissue from the first animal before the mice develop extensive lesions or die from the infection. An infectious agent, latent in the experimental animal, could thus be carried along during the successive passages, and increasing in virulence, could finally produce obvious disease. In this manner three different groups of investigators (54, 89, 103, 104) have encountered respiratory disease which differed from that seen with known viruses. They have further shown that the disease may be produced by repeated serial passage of lung tissue from apparently normal healthy mice, which indicates that a certain percentage of mice harbor the responsible agent. Two types of disease have been found, differing somewhat in course, host susceptibility, and production of immunity, although the possibility of immunological relationship between the respective viruses has not yet been determined. For the sake of simplicity, therefore, the two types will be described separately.

No instances of spontaneous illness due to either type of infection have been reported, although the viruses have been found in albino Swiss mice as young as 3 weeks of age, and in other albino strains obtained from a number of different sources. Small areas of spontaneous pulmonary consolidation occur in such animals with varying frequency: 1 to 2 per cent (104) and 35 per cent (54). The viruses must accordingly have a fairly wide distribution and a low virulence under natural conditions. Increase in virulence of the agents with successive intranasal passage would then account for the production of extensive and often fatal pneumonic lesions.

Pneumonia described by Dochez, Mills, and Mulliken (54), and by Gordon, Freeman, and Clampit (89).—This form of experimental pneumonia, first described by Dochez, Mills, and Mulliken (54), appeared after 1 to 9 intranasal passages. Clinically, the signs of infection were loss of activity, refusal of food, ruffled coat, and hunched posture, with the development of rapid, labored respirations as the disease progressed. Deaths began to occur after 4 to 7 passages, the mice succumbing 2 to 4 days after inoculation. The mortality rate was high; in fact, all mice (5 to 10 grams in weight) developing signs of infection died (89).

At autopsy the only significant lesions were found in the lungs. Early in the course of the disease, sharply demarcated, greyish-pink areas of con-

solidation were present in the apices or dorsal portions of the lung. The consolidation spread as the disease progressed and at death the entire lung might be involved, often having a uniform dark red or plum-colored appearance resembling the lesions due to the influenza virus (262). The microscopic picture was one of a patchy interstitial pneumonia, with mononuclear infiltration and varying degrees of hemorrhage and edema. Cellular exudate in the bronchial lumina consisted of mononuclear and polymorphonuclear leukocytes. The epithelium of the bronchi was well preserved in contradistinction to the necrosis and desquamation produced by the influenza virus. Non-inflammatory focal necrosis was present in the liver. A variety of organisms were cultured from the lungs in some instances, but none reproduced the disease.

The virus was present in the lung and in the liver (89) of infected mice, and passed Berkefeld N and V as well as Seitz filters. Mice were susceptible only to intranasal inoculation. No spread occurred by contact. In ferrets, administration of virus by the nasal route produced an elevation of temperature to about 105°F., occasionally associated with respiratory difficulty. Intratracheal inoculation of rabbits resulted in pneumonia, mediastinitis, and pericarditis, complicated, however, by the presence of secondary bacteria (54). The guinea pig was resistant. Protective serum was not produced in rabbits by administration of lung emulsions containing the virus, nor was active immunization of mice successful. The agent is apparently distinct from human influenzal virus, since mice convalescent from infection with the latter were fully susceptible to the murine virus. Further immunological studies are necessary, however, to achieve certain differentiation and identification of this agent.

Pneumonia described by Horsfall and Hahn *(**103**, **104**)*.—As already pointed out, this type of experimental pneumonia differs in certain important respects from that described in the preceding paragraphs. The disease was found to be latent in 3 of 8 different colonies of albino Swiss mice. Using 3 to 4-week-old animals, infection became apparent after 2 to 7 intranasal passages of the supernatant fluid from lung emulsion at an interval of 7 to 9 days, but not by rapid serial passage at 4 to 5 day intervals. The mice appeared well for 5 to 7 days, but then showed a decrease in activity and food consumption, loss of weight, ruffled fur, slow, deep, and sometimes labored respirations, and often cyanosis of the ears and tail. Death occurred from 8 to 14 days after inoculation. The morbidity and mortality rates varied with the amount of virus inoculated and the particular stock of mice employed.

The pulmonary lesions did not differ significantly from those described above. Consolidated areas varied in extent and were hilar in distribution with radiations outward along the bronchi. The histopathological appearance was essentially the same. In the great majority of instances (85 per cent) the lungs were sterile and such bacteria as were found had no etiological significance. It is of interest to note that pleuropneumonia-like organisms were isolated with ease and in approximately the same numbers from the lungs of normal mice as from those infected with the murine and influenzal viruses. These organisms did not reproduce the disease, nor did rabbit antiserum containing agglutinins neutralize the murine virus.

The virus was found to be strictly pneumotropic for mice and to increase in virulence with the first few serial passages. Routes other than intranasal failed to produce infection, and the virus could not be obtained from the brain after intracerebral inoculation, nor from the liver following intraperitoneal injection. Attempts to transmit the infection by contact were unsuccessful. Ten other species of animals, including rabbits, ferrets, guinea pigs, and *rhesus* monkeys, were resistant to infection. The virus has been cultivated in tissue culture with considerable loss in virulence.

Active immunity in mice was readily obtained by two intraperitoneal injections of living virus or by intranasal inoculation of amounts insufficient to produce death. All strains of this murine virus were identical immunologically and were easily distinguished from the human and swine strains of influenza by cross immunity and neutralization experiments. The virus was neutralized, however, by approximately one-third of 67 human sera, although in later experiments no association could be made with any of the respiratory diseases common in humans.

In suspensions of infected mouse lung the virus was inactivated at 56°C. in 30 minutes, and decreased in titer rapidly at room temperature unless protected by the addition of 10 per cent normal horse serum. No decrease in activity occurred when frozen and stored at −76°C. It was readily filterable through Berkefeld V and N filters but not through the Seitz filter. By the use of graded collodion membranes, its diameter was found to be approximately 100 to 150 mμ.

It thus seems probable that experimental pneumonias in mice, though similar pathologically, may be due to different viruses. The agents do not cause spontaneous illness and not all mouse stocks are infected with them. The original source is not known, but may be human (104), if neutralization of this virus is as specific as it is with other viruses. The primary importance of this disease to investigators, however, is its similarity to that pro-

duced in mice by the viruses of influenza (9, 80, 250, 262) and the possibility of mistaking its identity.

Inclusion bodies in the salivary glands and liver of the mouse.—Cellular inclusions have presented something of a problem to investigators for a number of years. Certain of them occur frequently in abnormal or malignant cells, but are artefacts due to intracellular necrosis and the action of ingredients in the fixatives (42). In the past two decades inclusion bodies of a different type have been found in cells of the salivary glands and liver of a number of animal species, including mice (68, 137, 273, 274, 168). They occur quite constantly in some stocks or breeds of mice. Transmission to normal young or adult mice is readily accomplished, is species-specific, and no bacteria or parasites are found in association with the bodies. Foci of chronic inflammatory cells are present in the affected organs. With the possible exception of an epidemic mentioned by Thompson (273, 275; see section on ectromelia), animals harboring them appear to be perfectly healthy. These characteristics suggest that an infectious agent of low pathogenicity is responsible for their production. The nature of the inclusion bodies—whether degenerative, metabolic, mutative, or infectious—is not known. Since the appearance of inclusions is concomitant with infection by many of the known viruses, however, it is logical by analogy to consider a virus as the causal agent here. Filterability, moreover, is reported in one instance (168).

Inclusion bodies in the salivary glands.—The incidence of salivary gland disease varies between 20 and 60 per cent in adult albino mice of certain stocks. Other colonies may be entirely free from the disease regardless of the age of the animals, but in general mice less than 1 month of age do not show the lesions. Spontaneous illness has not been described. The natural method of transmission has not been determined, but once a colony has been infected the disease continues for generations.

Histopathologically, lesions are found only in the salivary glands. Acidophilic intranuclear inclusions, usually large but of varying size, occur in acinar cells of the serous and mucous portions of the glands, occasionally in duct cells, and rarely in alveolar cells of the parotid. Such cells are hypertrophied and irregular in shape with granular, basophilic cytoplasm. The nuclear contents may be completely replaced or distorted by inclusions which are composed of minute spherules and are often surrounded by a halo. Scattered foci of mononuclear cells are present throughout the tissue, often without any apparent relationship to the affected acinar cells.

The disease may be transmitted to normal adult or young mice by inoculation of emulsions of the infected salivary glands or by filtrates of such emulsions. Negative results are obtained with other tissues. No clinical manifestations occur in adult animals and the virus localizes in the salivary glands regardless of the route of inoculation. Following intracerebral injection a mild meningeal reaction may result, with exudation of mononuclear cells and occasional inclusion bodies within cells of the cerebral tissue, endothelial cells of the choroid plexus, and mononuclear cells. In young animals (3 weeks of age) a fatal infection may be produced by intraperitoneal and occasionally by intracerebral inoculation (168). Death usually occurs in 3 to 7 days. Necrotic lesions are found most extensively in the liver, spleen, adrenals, lymph nodes, and subperitoneal tissue. Intranuclear inclusions are frequent in these tissues, but are not found in the salivary glands unless the animal survives for 8 days or longer. Experimentally, strains of mice vary in their susceptibility to the virus. Other species of animals are resistant.

The properties of the transmissible agent have not been fully investigated. It is destroyed by a temperature of 60°C. for 30 minutes. Filtration through a Berkefeld V filter has been accomplished.

Inclusion bodies in the liver.—Inclusions in hepatic cells are probably very uncommon since Twort and Twort (299) did not notice them in the course of some 12,000 postmortem examinations. Findlay (68), however, observed acidophilic intranuclear inclusions in the livers of all the mice of one strain (Clacton) obtained from a London dealer. They were not found in the livers of newly born mice. Transmission to a disease-free strain of mice was accomplished by inoculation of an emulsion of infected liver. This observation has been confirmed by Thompson (273, 275), who noted hepatic inclusions in 5 of 25 apparently healthy mice as well as during an epidemic which somewhat resembled ectromelia.

Factors Influencing the Production of Experimental and Natural Disease in Mice

The study of any infectious disease is best carried out in its natural host. For obvious reasons, however, an experimental study of certain diseases on such a basis may be impracticable if not impossible, and it is necessary to resort to a different species of animal. The disease thus obtained may or may not be similar to the original one, but it will be dependent, as is the natural disease, on at least three important variables: the microbe, the

environment, and the host. The extensive work in experimental epidemiology (276, 74, 5, 6, 181, 313, 314, 320, 94, 279, 92) well demonstrates the significance of these factors in natural diseases of the mouse, and numerous other observations attest their importance in artificial infections.

Microbic factors.—The type of disease resulting from the introduction of an infective agent into the body of the host is dependent on dosage, route of inoculation, and virulence of the agent. Thus, increase in dosage may alter the course from a benign subclinical infection to a rapidly fatal, overwhelming infection; or the subcutaneous route of inoculation may be entirely ineffective, whereas intracerebral injection produces a striking encephalitis. The role of virulence or pathogenicity is somewhat more difficult to assess. Strains of an organism obtained from different sources or in various stages of dissociation undoubtedly vary in their capacity to produce disease. Whether or not it is possible to alter the inherent virulence of a given strain by repeated animal passage is open to question, at least in the case of certain organisms, when all other factors are kept as nearly constant as possible (308, 192, 309, 310, 311, 312).

Environmental factors.—Various features of the environment—temperature, diet, season, number of animals per cage, and cleaning routine—alter the type of disease chiefly by their effect on host factors, and, to a less extent, on microbic factors. Thus, mice of the same stock reared on a bread and milk diet without obvious dietary deficiency were found to be more susceptible to mouse typhoid (329, 210) than those fed the more complete McCollum diet. Crowding of animals in a cage may affect the microbic factors by increasing the dosage or altering the route of infection if the organism is excreted by the inoculated animals.

Host factors.—When a group of mice, maintained under controlled environmental conditions, is given a standard dose of an infective agent, a certain number of them become ill and die, others recover, and still others may show no signs of infection. The relative proportion in each group will depend on the specific and nonspecific resistance of the host—a complex mechanism, the individual factors of which are not easily segregated and subjected to quantitative analysis. Considerable progress has been made in this direction, however, chiefly as a result of the stimulating investigations in the field of experimental epidemiology.

Specific resistance is considered to be an immunity acquired through previous contact with the infectious agent. That such specific immunity as a factor in resistance is operative in certain natural and experimental infections is generally accepted, but in others its relative importance in

comparison with nonspecific factors is questioned. Webster and Hodes (328) have recently demonstrated that highly susceptible mice are not immunized to a subsequent test dose by repeated, sublethal doses of mouse typhoid bacilli or St. Louis encephalitis virus given by a natural route. They further emphasize that reinoculation of survivors is not an adequate test of active immunity unless the animals employed are known to be " . . . at least 90 per cent susceptible to the test agent given by a normal portal of entry." An animal surviving the first dose by virtue of nonspecific resistance may withstand a second dose in the same manner without necessarily having an active immunity. Moreover, in such diseases as mouse typhoid and ectromelia, vaccination by the methods thus far employed may give some protection but does not confer a solid immunity (306, 205, 289, 92).

Nonspecific resistance appears to be a characteristic of the individual, dependent on heredity and probably on other factors as yet unknown, as modified by age and environmental influences. The degree of resistance varies among individuals in a single breed of mice as well as among different breeds (295, 277, 312, 315, 206, 208, 209, 90, 245). Because of this fact it is possible by selective inbreeding to develop stocks with a relatively high resistance or susceptibility to one infectious agent but not necessarily to another (316, 317, 289, 321, 322, 97, 326, 327, 323, 324). A study of hybrid and backcross generations (295, 90, 321, 324) indicates that resistance is dominant, but segregates independently of the sex and color factors. The responsible genetic factors are considered by Hill (97) to be multiple, since litters of long inbred lines may show more variation in reaction than can be ascribed to chance, whereas Webster (323) supports the theory of a single factor type of inheritance with possibly a number of small modifiers, since mortalities in succeeding generations showed no definite progress with selection. Both Hill and Webster have emphasized the need for extreme precautions in work of this kind in order to exclude the specific resistance of acquired immunity, either active or passive.

There are numerous observations, some of which are mentioned in the preceding pages (see also 183), to indicate that resistance varies with age, older animals in general becoming more resistant. Nonspecific anatomical and physiological factors (228) undoubtedly play a role, but there is also evidence that specific factors may be involved, since immature animals are less able than mature ones to respond to an antigenic stimulus by the formation of antibodies (20, 53, 175).

The above work is important not only because it aids in the selection and use of animals experimentally, but also because it points out many of the

important features to be considered in the prevention and control of natural disease in animal colonies.

Prevention of Disease and Control of Outbreaks

The application of general preventive measures is the only satisfactory way, in the absence of specific prophylaxis or therapeutics, to prevent the introduction and spread of natural disease in a mouse colony. Success will depend to a large extent upon the strictness with which the control measures are maintained. Some idea of the frequency with which spontaneous disease is encountered may be gained from the figures on occurrence given in the preceding sections. In addition, Greenwood and Topley (94) report that during 7 years the most important spreading diseases were due to *Salmonella typhimurium*, *S. enteritidis*, *Pasteurella muricida*, and *Erysipelothrix muriseptica*, whereas infection due to *Proteus morgani*, other types of Proteus, enterococci, and *Corynebacterium kutscheri* spread to a less extent.

It is apparent from the discussion in the last section that those procedures which increase the environmental and host resistance factors and decrease the microbic factor of dosage will thereby lessen the opportunity for the spread of infection. Of these, the environmental factors are probably most important, since they are most readily subjected to control and in themselves modify host resistance and dosage. An attempt will be made to present a description of the ideal physical equipment for the animal rooms, although experience has shown that departures from this in many respects can be made satisfactorily. The cleaning technique is modeled on that now in use in the Jackson Memorial Laboratory.

The animal rooms should be rodent proof, light, well ventilated with regulation of temperature and humidity, and so constructed as to permit washing of the walls and floor. This process is facilitated by a central drain and a rounded baseboard which obliterates the angle between the walls and the floor. The floor should be considered to be contaminated at all times and nothing placed thereon should be permitted to come in contact with cages, racks, tables, or other equipment of the room without sterilization. Low tables can be used to support clean cages during actual replacement of cages. Racks should be of simple metal construction set out from the walls to eliminate breeding places for vermin, and if possible suspended from the ceiling. They should be sufficiently spacious to permit arrangement of individual cages without contact between them. The cages

themselves may be of simple metal box- or pan-like construction with a detachable screen lid permitting replacement of water bottles and food without removal of the lid. Food and bedding are best stored in metal containers, bins, or special rooms protected from vermin and stray rodents. Each cage as a unit should house the smallest number of mice consistent with the total number and available space. Breeders are best kept in separate cages in a separate room.

Washing of the rooms, racks, and transfer of cages should be carried out at least once a week. The attendant is best garbed in a coverall or gown which can be laundered, and should scrub and dip his hands in disinfectant between sections of racks while transferring animals from dirty to clean cages. In a separate room the cages and water bottles are cleaned, washed, and sterilized, preferably by steam. If chemical sterilization is employed, a sufficiently long period of contact must be allowed to insure effective action of the germicide. Sterilized wood shavings are most satisfactory for bedding, and may be placed in the clean, dry cages before they are removed to the animal room for the next cleaning. By following this cleaning technique, dust in the animal room itself is reduced and disposal of waste becomes a simple matter.

A nutritionally complete diet may be prepared on the basis of the McCollum or Steenbock formulas or their modifications (329, 321). Adequate diets are also available commercially. Under ordinary circumstances, sterilization of the food is not necessary. Except in special instances, little can be accomplished by attempting to alter the specific host factors.

Before being added to the general stock, new mice should be kept in quarantine for at least 3 weeks, distributed in separate cages containing 4 to 6 mice each. Postmortem examinations, with cultures, should be made on all dead animals. If infection is recognized, the cage-mates must be killed. Should no cause for the death be found, the other animals in that cage are watched for an additional 2 or 3 weeks. A second death is an indication for destruction of the remaining animals in the unit; otherwise they may be considered to be normal.

Measures similar to quarantine should be taken in the event of an outbreak of disease in the general stock. At the first appearance of the disease the room should be rigidly isolated and the diseased mice cared for only by attendants who have no contact with normal animals. Depending on previous conditions, the animals should be redistributed into the smallest possible number per cage unit. A specific death is then an indication for the destruction of all the mice in that unit. If the disease is very extensive,

it may be necessary to kill all the animals, but in all probability the above procedure will prevent an extensive epidemic, or at least permit a number of survivors from which the stock can be rebuilt. Since in a number of diseases the carrier state may be persistent, care must be taken in adding new susceptible mice or in augmenting the number of animals per unit, until examination of a sufficient sample of apparently normal mice and of those dying sporadically reveals no evidence of the disease.

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Chapter 13

CARE AND RECORDING

By John J. Bittner, *Roscoe B. Jackson Memorial Laboratory.*

The salient features in the care of mice are probably similar in most laboratories. The majority of investigators naturally consider that the methods they use are superior to those employed by others. In some respects they may be correct as the system one must apply is often determined by unusual circumstances which only experience may correct. A stock of mice maintained in a satisfactory condition by one worker may die out under the care of a second with conditions practically identical. There is also in the management of mice some indescribable "knack" which some have and others apparently lack. There is no indication that this is due to a lack of interest on the part of the latter individual; it is more likely to result from too much attention and handling of the animals.

The method to be described for the care of mice is that followed at the Jackson Laboratory with satisfactory results.

The mouse boxes (Fig. 171) are made of wood and measure 12″ × 12″ × 6″. The bottom is covered by ¼″ plywood. The front, back and sides of the box are made from ½″ stock and the center partition is ¾″ thick. The thick center partition gives more room for the covers to overlap and the life of the box is lengthened as it is through this board which the mice are most likely to gnaw. The boxes may be painted or may be dipped in a solution of equal parts of turpentine and linseed oil containing dryer. Lionoil may be substituted for the linseed oil.

Wooden boxes have some advantages over metal or wire cages, the most important being that the mice are warmer than they would be in metal boxes and are not subjected to drafts. Also, less light penetrates the boxes thus giving a more natural habitat for the rearing of young. The initial cost is considerably lower than for the other types, but replacement must be made more often.

The stock for the covers measures 1¼″ × ¾″ with the pieces cut so that part fits down into the box and the flange overlaps the side of the box to support the weight of the cover, the food hopper and the water bottle. The

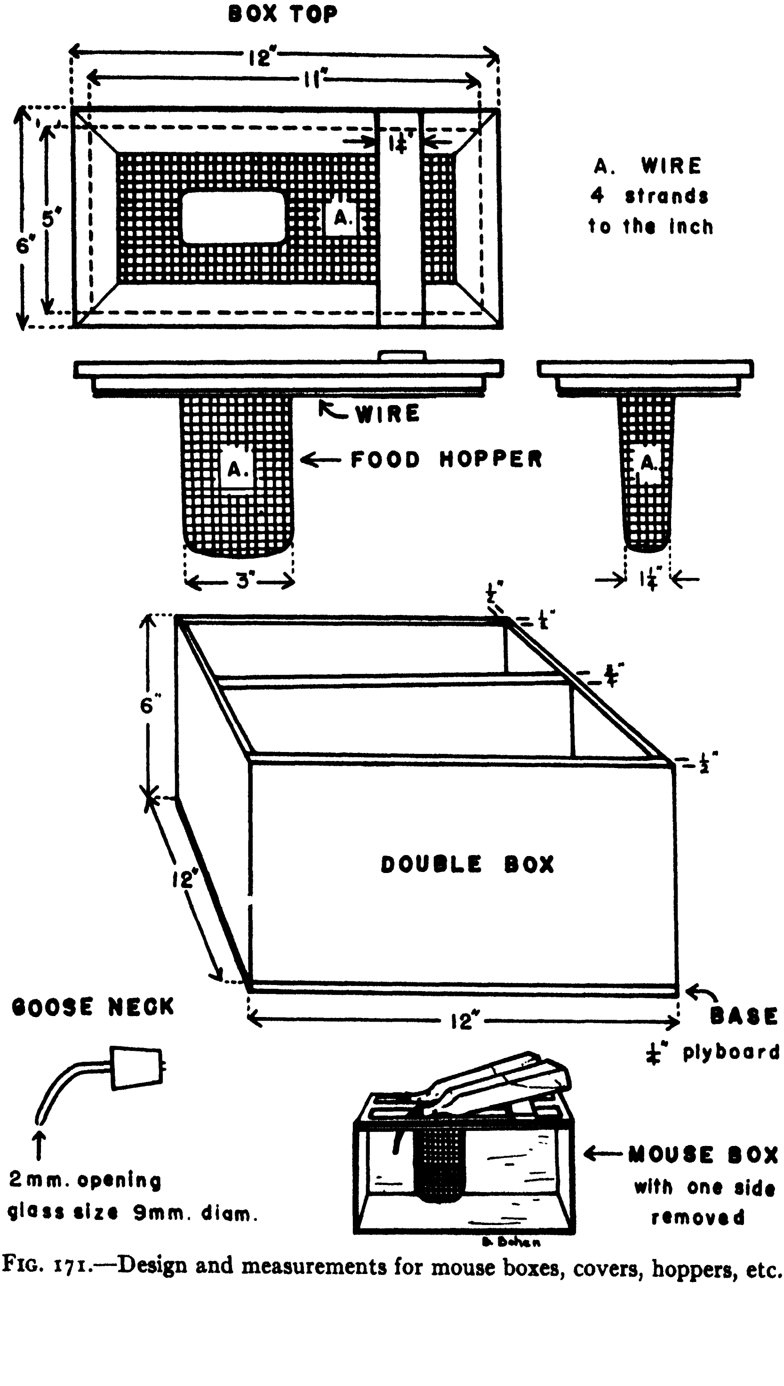


FIG. 171.—Design and measurements for mouse boxes, covers, hoppers, etc.

wire is nailed on the under surface of the frame to prevent the mice from chewing the cover and the edges of the box.

A cross-bar near the front on the covers supports the water bottle (16 oz.) in a slanting position. A bent glass tube passing through a rubber stopper extends from the water bottle down into the pen. The end of the gooseneck is partially closed to form a nipple from which a drop of water hangs. There is no leakage from the water bottle unless the opening in the tube is too large. Ordinarily the bottles need to be filled no oftener than once a week.

A hole is cut in the wire of the cover to receive the wire food hopper which measures, at the top, 3×2 inches. At the bottom of the hoppers the measurements are $3 \times 1\frac{1}{4}$ inches. The wire from which the hoppers are made has four wires to the inch and the parts are sewed together with fine copper wire. The upper parts of the hoppers are bent so that they interlace with the wire of the cover to hold the hoppers in place and prevent openings through which small mice might escape. The hoppers extend to within an inch from the floor of the cage and hold sufficient food to last six mice one week.

Several commercial foods in pellet form are available for use in hoppers of this type.

The type of rack which one uses is important from the standpoint of cleanliness and the control of vermin. Metal racks are more satisfactory from this standpoint than are wooden shelves. The clearance between shelves should be at least 11 inches. The shelves may be 12 inches or 24 inches wide to provide space for one or two rows of boxes. In small quarters the shelf space may be increased considerably by careful planning and the use of certain types of racks.

All boxes containing mice should be changed at least once a week. The clean boxes should contain shavings or sawdust and a small amount of cotton for bedding. Some stocks require shredded paper in place of cotton. The soiled bedding in the used boxes should be entirely removed after which the boxes should be thoroughly sterilized and dryed before they are used again. The water bottles and covers should be washed at intervals.

The mice are marked by a series of holes and notches on the ears (Fig. 172). The units are recorded on the right ear, the tens on the left ear. Number one, two and three are represented by holes at the front, top and back respectively; four, five and six each by a single notch starting from the front of the ear; seven is represented by two notches close together at the front; eight by two notches at the top and nine by two notches at the back

of the ear. By this system it is possible to number, on the ears, from 1 to 99 for individual identification. The complete serial number of each animal is kept on the individual card.

The animals are marked when they are weaned and breeding pens are usually made up at this time. Each side of the boxes has sufficient space for six adult animals and thus five females may be mated to each male in strains where the animals show high fertility. Matings in inbred strains of mice are always brother × sister unless a son is mated to its mother or a daughter to its father. Cousin matings should *never* be made if one wishes to maintain a homozygous stock. A practice which will save considerable time eventually is to keep a small pedigree chart for every strain on which every individual is recorded which has been mated. The development in any animal of any desired character or condition should be noted on this chart. After every few generations the best line may be selected from which to continue the stock. Litters or sub-lines which are not wanted should be discontinued. Unless some method for selecting the matings is used, the strain may eventually consist of sub-lines which differ markedly from each other. If the development of different sub-lines is desired the chart will aid in assuring the worker that all lines are being continued.

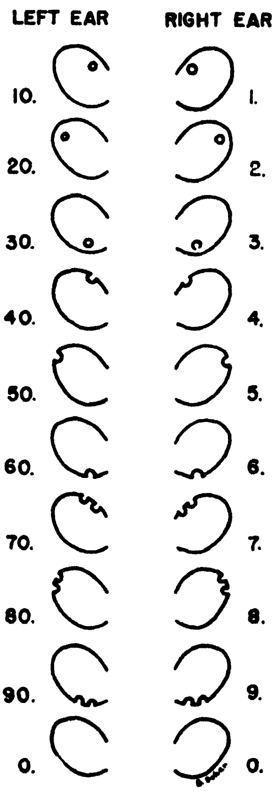


FIG. 172.—Ear markings for individual identification; units on right ear, tens on left ear.

On the individual female card are recorded the number, color and sex of each mouse. Space is also provided for the strain or line, the inbred generation, date of birth, death, development of spontaneous tumor, age at death or development of tumor. The pedigree of the animal may be written at the bottom of the card and the mating and breeding box number inserted. Space has been provided to enter data for twelve litters. In successive columns, from left to right, may be given for each litter: the generation, ledger number, date born, number born, born dead, weaned, females, males, age of mother at the birth of each litter and the number of days between litters. These cards may be altered to catalogue any information that a worker may want in his investigations. (Sample card will be supplied on request.) The male card may be a ruled 4 × 5 library card

on which may be listed the data regarding the male and the numbers of the females to which it has been mated.

In addition to the individual cards a serial ledger should be maintained in which all the litters are entered. A separate ledger may serve for each stock or a continuous one for all stocks.

Pregnant females should be given an individual pen in which to have and rear their young. Experience will show that more and better young will be raised to weaning age if this system is followed. The female's individual card should be changed from the breeding pen file to that corresponding to the new box when she is separated from the male. The new box number may be noted on the male card to assist in locating the female at any time before she is returned to the home pen.

On the date of birth of a litter all the desired data should be recorded on the individual card. The recording in the ledger may, if desired, be delayed until the young are weaned.

Consideration should be given to the following details for the satisfactory care of mice.

A well heated and ventilated room should be available. The temperature should be approximately 72° at all times. If an automatic ventilating system is not used, care should be used in ventilating the animal room by means of windows. Avoid sudden changes in temperature and direct drafts on the mice as they easily contract pneumonia.

Take rigid sanitary precautions in the care of the boxes, bottles, goosenecks and racks. If the water bottles are all filled at one time the sterilizing of the goosenecks is advisable to prevent the spread of disease from one box to another. Covers and hoppers should be cleaned at regular intervals.

Food and water should be before the mice at all times. The selection of the food may only be determined by experience as some strains will do better on one diet than another. Soiled food remaining in the hoppers when the boxes are changed should be discarded.

Do not place too many mice in a single pen. Overcrowded mice often become infested with mites or lice. These may be controlled by periodic dusting with powdered tobacco or a mixture of 1 part of derris root powder and 3 parts of talcum powder (it may be necessary to sterilize the ingredients before they are used).

To obtain and maintain inbred strains, mate only brothers and sisters. Mark all animals used for experimental or breeding purposes and make use of individual cards and a ledger for complete records.

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